GENETICS

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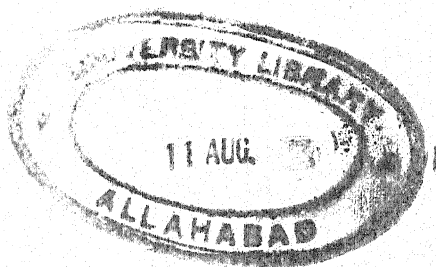
HENRY HOLT AND COMPANY • NEW YORK

PREFACE

$\mathbf{I}$N THE present text emphasis has been placed on modern genetics. The author has attempted to make the student feel that genetics is a growing science, that important developments are now taking place. The definition often given of genetics as the study of heredity and variation is no longer adequate. Genetics, of course, deals in addition with the origin and evolution of the germ plasm, with its physical and chemical constitution, with its interactions with the environment, with its spread and distribution, and with its influence on development. Genetics in the modern sense might therefore better be defined as the science which concerns itself with the study of the germ plasm. The present treatment considers all the subdivisions of genetics as thus defined. At the same time it is realized that a clear understanding of classical genetics is necessary before the more modern developments can be entered into.

Emphasis too has been placed on principles rather than on details of a highly technical nature, and an attempt has been made to combine clarity and interest with conciseness. In preparing the manuscript the author constantly asked himself, "Could I understand this if I were the student?" Yet no important subject matter was omitted simply because it was difficult. The author does not mean to imply, however, that he has completely covered all the literature. This would be impossible, within the limits of a textbook, for a literature that has become as vast as that of genetics.

Nowadays it is hardly necessary to point out that a knowledge of the physical basis of heredity is absolutely essential to a sound understanding of genetics. In the present treatment, therefore, the physical basis is presented at the very start. In the naming of genes the "presence and absence" nomenclature has been completely abandoned in the present text. The black mouse is b, not a (the

absence of agouti). But the student is told of the older systems of nomenclature in order that he might recognize and understand them in the literature, and at the same time he is given the reasons for preferring the new nomenclature.

The chapter on heredity and development attempts to represent the gene in its proper relationship to organizers, fields, gradients, and hormones. The problem of cytoplasmic inheritance is included in this chapter, but an attempt has been made to sift out the essential facts from the conflicting evidence on the subject.

It should perhaps be explained that the writer is fortunate in having been engaged in research in genetics at Columbia University from 1911 to 1916, and in having occupied one of the laboratory desks in the "Drosophila room" between 1911 and 1913, when many of the sensational discoveries in connection with the chromosome theory were being made. Hence in a number of places in the present text he has been able to draw upon this personal experience of the events that occurred during those historic times.

The author is greatly indebted for many helpful suggestions to Mr. Joseph D. Thomas of the English department of The Rice Institute, and he is likewise indebted to his wife, Luolin Storey Altenburg, for making the drawings and diagrams and for help with the manuscript.

                                                                E. A.

The Rice Institute,
Houston, Texas,
January 28, 1945.

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1. THE PHYSICAL BASIS OF HEREDITY

IT IS a familiar fact that a child resembles his parents. Yet the only physical connection between a father and his child is a sperm cell. It is obvious therefore that this microscopic body must contain all of the hereditary capacities of the father. The egg in turn must contain those of the mother. What gives the sperm and egg their remarkable qualities?

Chromosomes.—The sperm and egg cells, like all other cells, contain a nucleus, and it is to the nucleus that we must look for the structures which are concerned with inheritance.

Now ordinarily there is no particular structure definitely visible in the nuclei of the sperm and egg cells. But it is known on indirect grounds that the nuclei contain rod-shaped bodies called *chromosomes* (Fig. 1). It is these bodies that constitute our inheritance. They get their name from the fact that they take certain stains more heavily than do other parts of the cell, the term chromosome being derived from the Greek *chromos* (color) and *soma* (body). The human sperm cell contains twenty-four chromosomes; so does the egg cell. Thus the fertilized egg formed by the combination of the two contains forty-eight chromosomes.

We have been referring to man. But all other species of animals and plants contain chromosomes, though not necessarily the same number; and in all forms of life the chromosomes are the bodies that link one generation to the next and that cause the offspring to conform to their parents. In some manner, at present not understood, the chromosomes determine the course of development, and thus they cause the offspring to resemble their parents. The fact that the chromosomes come from both parents explains why the offspring resemble both parents; and the further fact that the same number comes from both explains why each parent has an equal influence in heredity.

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It is important in this connection to note that the chromosomes of the sperm and egg cell correspond to one another in a one-for-one manner. Thus in Fig. 1 a sperm cell is shown with a long, a medium, and a short chromosome and an egg cell with the same three kinds. The fertilized egg therefore contains a *pair* of each kind. The members of a given pair are known as *homologous* chromosomes.
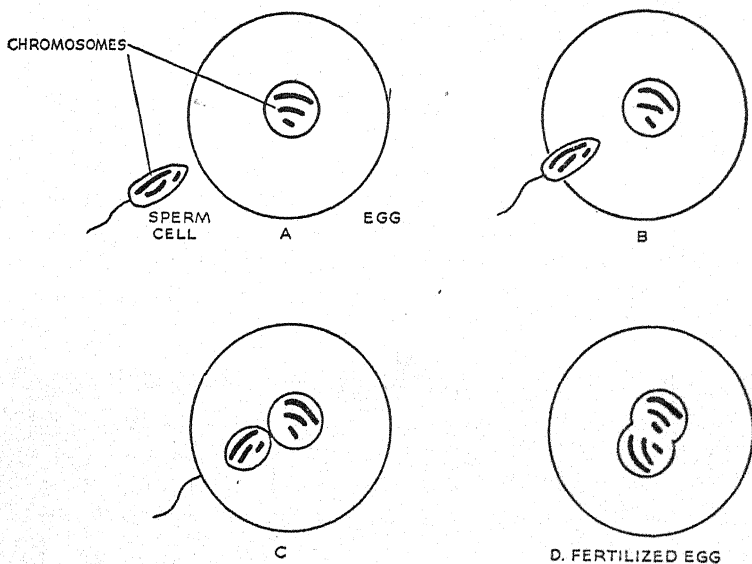
Fig. 1. Chromosomes and fertilization.

All the chromosomes in either a sperm cell or egg cell are referred to as a *set*. A cell which has one set of chromosomes is said to be *haploid;* a cell with two sets is said to be *diploid.* The sperm and egg cells, then, are haploid; the fertilized egg, diploid.

Mitosis.—When the process of development begins, the fertilized egg divides into two cells; but before it divides, the chromosomes first appear (Fig. 2a) and each of them splits lengthwise into two (Fig. 2b). They then line up in the middle of the cell and become connected with fibers that come from opposite "poles" of the cell. The split halves of each chromosome next pass to the opposite poles (drawn there according to some biologists by the contraction of the fibers, according to others by electrical or other

physical forces which themselves produce the fibers). The cell now divides into two with the result that each new cell has a split half of every chromosome present in the fertilized egg. It is important to notice that the chromosomes still run in pairs, just as they did in the fertilized egg.

The chromosomes in the new cells grow to their original sizes and they furnish the substance that essentially constitutes the nuclei of these cells. When the new cells prepare in turn to divide, the chromosomes first divide. Each cell then divides in essentially the same way as did the fertilized egg. Thus four cells are formed,
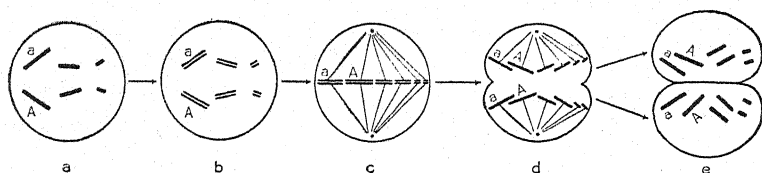
Fig. 2. Miotic cell division, as represented by the division of the fertilized egg. One of the chromosome pairs (the long pair) has been labelled (aA).

each with forty-eight chromosomes (in man). The process of cell division is repeated until millions of cells are formed, each with forty-eight chromosomes. Note that the chromosomes in the mature body are all the "descendants" of those originally present in the fertilized egg. Note too that their number, forty-eight for man, is maintained in every cell so long as cell division is preceded by chromosome division; for one cell becomes two through division and at the same time the one group of forty-eight chromosomes becomes two groups of forty-eight, one for each new cell.

Cell division preceded by the splitting of each chromosome is known as *mitotic cell division* or simply as *mitosis.* The term *mitosis* is derived from the Greek word for thread (*mitos*) and refers to the threadlike appearance of the chromosomes very early in cell division, at the time they split.

The Details of Mitosis.—Names have been given to the various stages of mitosis. The early stage, when the chromosomes are emerging from the nucleus and passing to the middle of the cell, is known as the *prophase.* The stage in which they are in the middle of the cell is known as the *metaphase.* The stage in which the split halves are passing to opposite poles is the *anaphase.* The final stage, when the split halves have arrived at opposite poles, is the *telophase.*

During the time that a cell is not dividing it is said to be in the *resting stage* or the *interphase* (Fig. 3a). As a rule no chromosomes are visible in the nucleus during the resting stage, but the nucleus does contain a stainable material from which the chromosomes are derived, known as the *chromatin*. This has no definite visible structure; it forms a sort of spongework. Lying close to the nucleus is often found a small granule known as the *centrosome*, concerned

A. RESTING STAGE
(OR INTERPHASE)

B. PROPHASE
(EARLY, CHROMOSOMES
CONDENSING OUT)

C. PROPHASE
.(CHROMOSOMES SPLIT)

D. METAPHASE

E. ANAPHASE

F. TELOPHASE
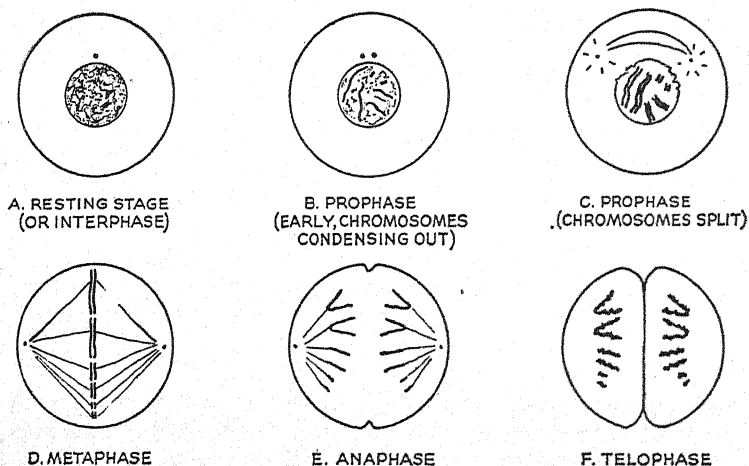
FIG. 3. The details of mitosis.

with cell division. Centrosomes are found in the cells of animals and lower plants but not in those of higher plants (such as a rose, lily, or onion plant). Undoubtedly the cells of higher plants contain material that is the equivalent of a centrosome but this material is not concentrated to a point.

When the cell prepares to divide by mitosis the chromatin resolves itself into the chromosomes (Fig. 3b and c). These are at first very thin and long, or threadlike, in appearance. It is during this time that they divide lengthwise into two. The nuclear wall then breaks down and the chromosomes are free to wander through the cytoplasm (the living substance outside the nucleus). The chromosomes now pass to the middle of the cell, as already mentioned. While they are doing so, they get shorter and thicker and assume the rod-shaped appearance typical of chromosomes. All this happens during prophase. At the same time the centrosome

divides into two (in animals and lower plants). The two daughter centrosomes then separate and pass to opposite poles of the cell. While they are doing this fibers develop between them and attach themselves to the chromosomes. The fibers as a group form a spindle-shaped figure and are known as spindle fibers.

The chromosomes have now arranged themselves in a circle in the middle of the cell and this takes us to the metaphase (Fig. 3d).

A. RESTING STAGE   B. PROPHASE   C. METAPHASE

D. ANAPHASE   E. TELOPHASE
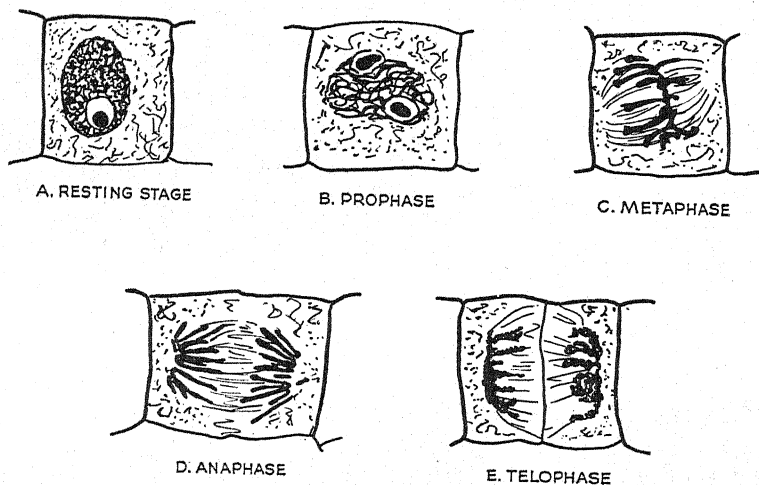
FIG. 4. Cell division in an onion root tip. (From Wilson, *The Cell in Development and Heredity*, by permission of The Macmillan Company, publishers.)

We can look at the circle in edge view and then the chromosomes seem to be arranged in a straight line in the middle of the cell. The plane in which the chromosomes lie at metaphase is known as the *equatorial plate*. One half of each split chromosome is on one side of the equatorial plate, the other half on the other side. The spindle fibers, chromosomes, and centrosomes form a body which has been named the *spindle figure*, in reference to its shape.

The anaphase begins the moment the chromosomes start to separate to opposite poles, and this stage passes gradually into the telophase or final stage of cell division (Fig. 3e). During the final stage in mitosis the chromosomes seem to send out branches from their sides. The branches connect and interlace, with the result that the boundaries of the individual chromosomes are no longer visible. Thus the chromatin is formed. At the same time a nuclear wall

surrounds the chromatin and so the nucleus of each new cell is completed.

Figure 4 shows cell division as actually seen in a growing root tip of an onion. At the metaphase stage the chromosomes dangle from the middle of the cell. Nevertheless, their split halves are drawn to opposite poles, as shown in Fig. 5. This is due to the fact that at metaphase the split halves are on opposite sides of the equatorial plate at the points of spindle fiber attachment.

EQUATORIAL PLATE

A. METAPHASE   B. ANAPHASE (EARLY)   C. ANAPHASE (LATE)
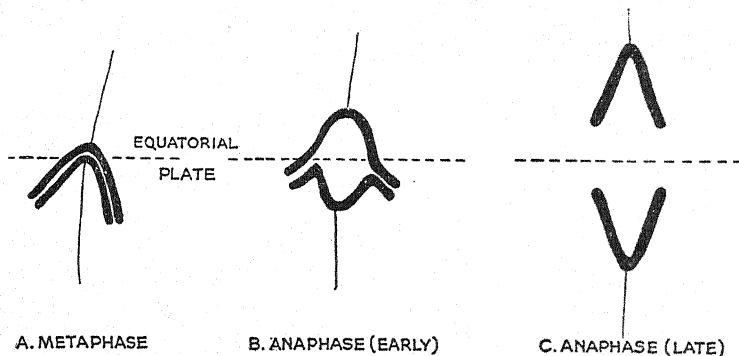
FIG. 5. The distribution of the split halves of a chromosome in an onion cell.

The Reduction Division.—What has just been said regarding cell division applies to ordinary cells, such as those that give rise to the brain, skin, muscles, and so forth. These cells all have the same chromosome number as the fertilized egg—forty-eight (in man). But the sperm and egg cells have only twenty-four chromosomes (in man). This is half the number in ordinary cells and is known as the *reduced* number (or the haploid number). It is necessary that the sperm and egg cells should have the reduced number, for if each had forty-eight chromosomes, like ordinary cells, then by their union they would form a fertilized egg having forty-eight plus forty-eight or ninety-six chromosomes, and so there would be a doubling of the chromosome number in this and every later generation.

The reduction in chromosome number in the sperm and egg cells is brought about through a special type of cell division known as the *reduction division*. This takes place in the reproductive organs. In cells that undergo this particular type of division the chromosomes do a very unusual thing. They come together in pairs

(Fig. 6). While they are still paired they split as they do for an ordinary mitotic cell division. Next the pairs move to the middle of the cell. The two members of each pair then separate to opposite

THE RE-
DUCTION
DIVISION

THE EQUA-
TION
DIVISION

THE MATURE
SPERM
CELLS
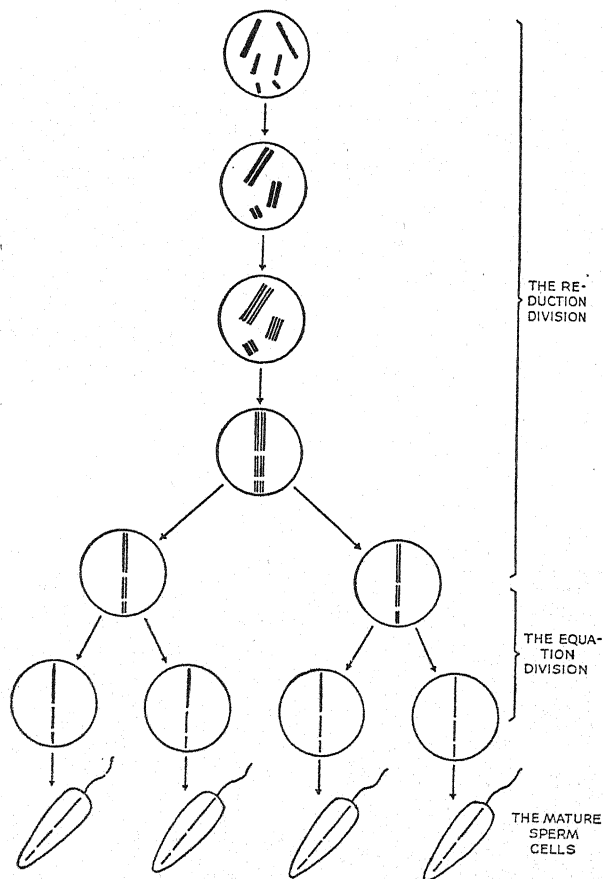
FIG. 6. The reduction and equation divisions (in the testis).

poles, each chromosome still consisting of two split halves. Next the cell divides into two, the plane of cell division passing in between the two poles. In this way the chromosomes number is halved. Each cell formed by the division contains just one member of each chromosome pair (though each chromosome consists of two split halves).

The cells formed by the reduction division do not develop directly
into the sperm and egg cells. Instead, they undergo one further cell
division often referred to as the *equation division*. At this division
the split halves of each chromosome separate to opposite poles, and
the plane of cell division then passes between the split halves. Thus
each resulting cell now contains one representative of each chromo-
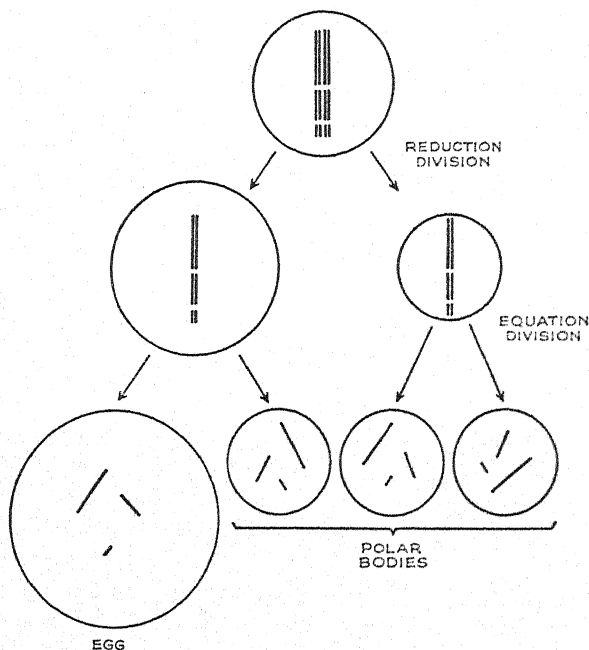
FIG. 7. The reduction and equation divisions in the ovary.

some pair. The equation division results in no further reduction in
chromosome number. It simply separates the split halves of each
chromosome. Hence it is called the equation division.

As a result of the reduction and equation divisions four cells are
eventually derived from each cell that undergoes the reduction
division. In the testes all four develop into sperm cells. In the
ovaries only one develops into an egg; the other three become
functionless and stick to the side of the egg, forming small cells
known as *polar bodies* (Fig. 7).

In considering the formation of the sperm and egg cells, it is
often convenient to ignore the equation division and to treat the

reduction division as though it involved simply the pairing of two unsplit chromosomes and led directly to the formation of the mature reproductive cells, as shown in Fig. 8. This is just a short-cut method of representation. The net result of the reduction and equation divisions is that members of a chromosome pair are separated and go to different reproductive cells. The short-cut method gives this result.

Why the reduction division should be followed by another division and why the polar bodies should be formed in the female are questions of detail that need not concern us here. The important thing for the present is that the mature reproductive cells—the sperm and egg cells— have the reduced number of chromosomes.

Summary of Chromosome Distribution over the Entire Life Cycle.—Figure 9 shows the distribution of the chromosomes from the time the individual begins his existence as a fertilized egg until the time that he is mature (the frog being chosen as an example). In this figure the sperm and egg cells are shown each with three
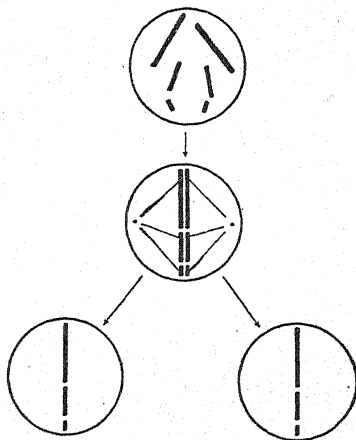
FIG. 8. The reduction division, short-cut method representation.

chromosomes (though in the frog the number is actually greater). The fertilized egg therefore has three pairs of chromosomes, or six in all. The fertilized egg divides and forms the first two cells, each with six chromosomes (three pairs). The first two in turn divide and form four cells, each with six chromosomes. The four divide and form eight cells, each again with six chromosomes. As development proceeds, a mass of cells is formed constituting the young embryo, and each cell still has six chromosomes. As the embryo grows older its cells differentiate and form various tissues, such as the lining of the gut, the muscles, and so on. These continue to have six chromosomes in each of their cells. The young animal also forms its reproductive organs (testes, in the case of the male). These at first contain six chromosomes in each of their cells. But as
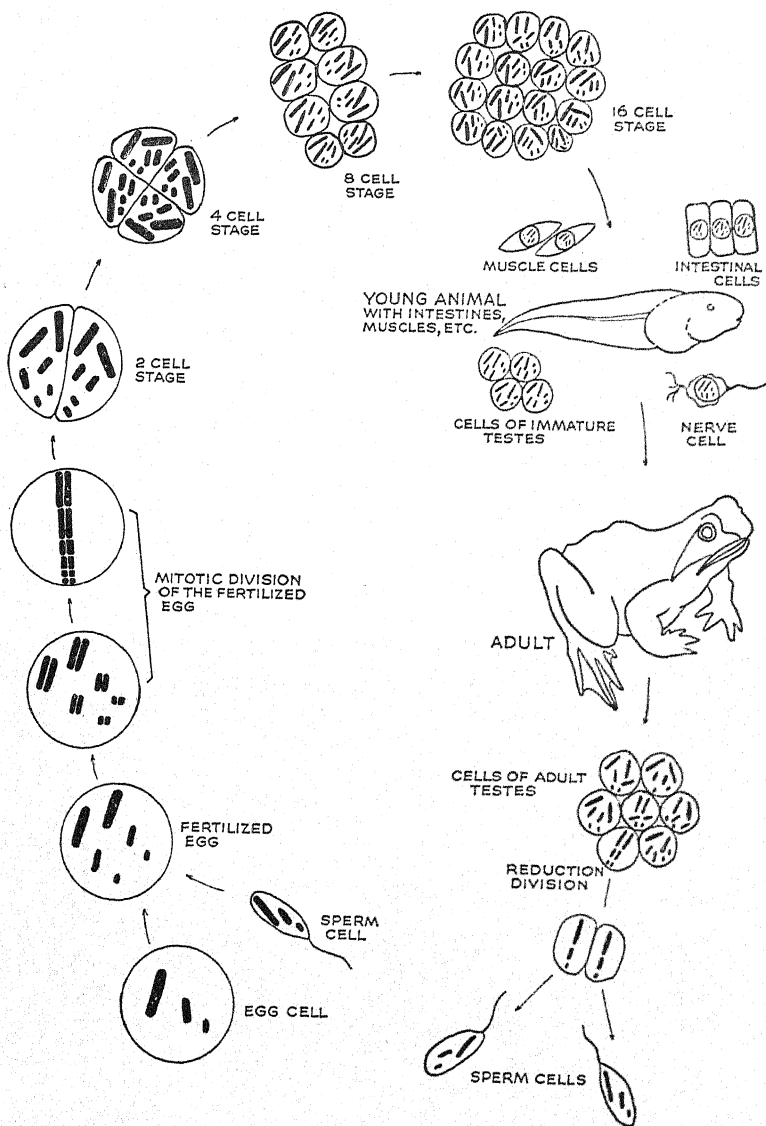
FIG. 9. The distribution of the chromosomes over the entire life cycle of an animal.

the animal grows older, the reduction division takes place. The chromosome number is now reduced from six to three, and each of the ripe reproductive cells contains three chromosomes.

The Point of Spindle Fiber Attachment (Centromere).— There is a definite point on the chromosome to which the spindle fibers attach themselves, known as the *spindle fiber attachment*, or *centromere*. Its precise location may vary from one chromosome to another, but for a given kind of chromosome it is always in the same place. Sometimes it is located in the middle of the chromosome (median attachment); sometimes it is somewhere between the middle and one end (sub-terminal attachment). It is seldom if ever at the very end, though it may sometimes appear to be, and is then loosely referred to as terminal.

The particular shape of a chromosome as it is being dragged to its pole during anaphase depends on the location of the spindle fiber attachment. If this is median, then the chromosome is V-shaped because both ends of the chromosome lag behind the middle (Fig. 3e). On the other hand, if the attachment is almost terminal the chromosome is straight; and if it is sub-terminal, it is J-shaped.

When a chromosome divides lengthwise its spindle fiber attachment also divides, with the result that each chromosome-half has its own spindle fiber attachment. As the two chromosome halves are being dragged to opposite poles of a dividing cell (during anaphase) they have the same shape because the spindle fiber attachments have corresponding positions in the two halves (Fig. 3e).

Evidence for the Individuality of the Chromosomes.—At the end of cell division (during the telophase) the chromosomes at opposite poles of the dividing cell are symmetrically disposed with respect to one another, as just explained (Fig. 10a). After cell division is completed two "sister" cells are formed adjacent to one another. When two sister cells in

a. CHROMOSOMES ENTERING RESTING STAGE   b. CHROMOSOMES LEAVING RESTING STAGE

Fig. 10. The symmetrical arrangement of chromosomes in sister cells, as seen in the early cell divisions of *Ascaris* (a parasitic worm). (From Boveri, in *Archiv fur Zellforschung*.)

turn prepare to divide, their chromosomes are still symmetrically disposed towards one another (Fig. 10b). We could account for this

fact most simply by assuming that the chromosomes persisted during the resting stage and that they remained in their positions in the resting nucleus from the end of one division to the beginning of the next. This then indicates that the chromosomes maintain their identity from one cell division to the next.

The fact that the chromosomes persist in number from one cell division to the next is in itself evidence that the chromosomes themselves persist. They also persist in size and shape. Moreover, if an extra chromosome happens to get into a fertilized egg, it continues to make its reappearance with each cell division and to maintain its characteristic size and shape. Even fragments of chromosomes persist. If on the other hand a particular chromosome, or even part of one, is lacking at the start, it is never replaced; neither is a segment, if knocked out of a chromosome, ever replaced. In some species the chromosomes can actually be identified in the resting nuclei and are known as *pro-chromosomes*.

We might summarize by saying that the chromosomes maintain their individuality from one cell division to the next as shown by (1) the emergence of the chromosomes from the resting nucleus in the same configurations as those in which they entered it, (2) the persistence of the chromosomes in number, size, and shape from one cell division to the next, and (3) the occasional visible persistence of chromosomes in the resting nucleus in the form of pro-chromosomes.

The Chromonema.—What does the inside of a chromosome look like under high powers of the microscope? Ordinarily we cannot see any particular structure within a chromosome, but at certain times in cell division and in favorable material a chromosome can be seen to contain a spirally wound-up thread. This is
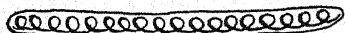
Fig. 11. The chromonema.

known as the *chromonema* (Fig. 11). Along the length of the chromonema granules can be seen (not shown in Fig. 11), reminding us somewhat of beads along a string. The part of the chromosome that we ordinarily see under the microscope is only its shell; the important part is the chromonema. At times the shell is discarded and the chromonema becomes unwound. Chromonema and chromosome are then one and the same thing.

It will be recalled that when the chromosomes condense out of the chromatin at the beginning of cell division, they at first appear

as long thin threads. At this stage they consist of the more or less unraveled chromonema. They then condense into the rod-shaped form that chromosomes ordinarily have. This they do by winding themselves up spirally and surrounding themselves with a shell.

The lengthwise division of a chromosome into two takes place when it is in the chromonema stage. After division the two resulting threads wind themselves up spirally and each surrounds itself with a shell, but the two threads and their shells remain side by side for a while. This gives the chromosome the appearance of a rod-shaped body that has split itself into two, though it is really the chromonema that has divided longitudinally.

Genes.—Chromosomes contain minute bodies known as *genes*. These are the *ultimate units of heredity*. Genes are arranged in linear order in a chromosome, like beads on a string. The important part of a chromosome is the chromonema and this is the part which contains the genes in linear order. It has been estimated that there are several thousand genes within one chromosome. This makes genes very small and ordinarily we cannot see them, even under the highest powers of the microscope. It was stated above that along the length of the chromonema granules can be seen, and perhaps these are the genes, though we are by no means certain that they are.

But if we cannot see genes for certain, then how do we know that they exist? The evidence for the existence of genes is for the most part indirect and is based on breeding experiments. The student of heredity is in somewhat the same position with respect to genes as the physicist with respect to atoms. Nobody has ever seen atoms— they are too small to be visible. Yet the physicist and chemist know that there are such things as atoms because the experimental evidence demonstrates this. Chemical reactions could not be explained without atoms. So with genes. Breeding experiments demonstrate their existence, as we shall see later, when we examine the experimental evidence.

We have little information about the chemical composition of a gene or of its intimate nature in general. We do, however, know that genes have a very remarkable property—the *power of growth*. They can take material unlike themselves and convert it into their own substance, and thus they grow. When they reach a certain size they divide into two; or possibly one gene builds up another one next to itself. Thus they multiply. The growth and division of a

chromosome into two is merely the expression of the growth and division of its individual genes.

Mutations.—Genes ordinarily do not undergo any permanent change from one cell division to the next or from one generation to the next. They are relatively stable. But very occasionally a gene changes. As a result a new type of plant or animal might arise, such as a pea plant with white flowers instead of the normal red. A permanent change in a gene is known as a *mutation*, and the new type of plant or animal as a *mutant*.

A mutation has little or no effect at the time it occurs, because it is at first confined to one cell. But if the mutation takes place in a
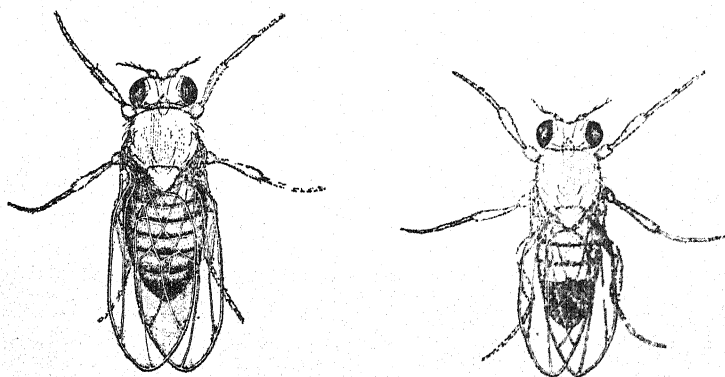
FIG. 12.   *Drosophila melanogaster* (the fruit fly).   This small insect is very favorable for the study of genetics.   (Drawing by Vera Storey Mandeville.)

sperm or egg cell or in a cell that is ancestral to a sperm or egg cell, the mutated gene might be passed on to an offspring and from this eventually a new race might be derived which shows the mutant trait, such as white flowers instead of red.

Mutations have been observed in many forms of life. Especially favorable for the study of mutations is a small insect known as the fruit fly, *Drosophila* (Fig. 12). This insect is extremely easy to breed in the laboratory in large numbers. All that one need do to breed it is to put some banana in a milk bottle, add a male and female Drosophila, and stopper the bottle with some cotton. After mating, the female lays her fertilized eggs on the banana. In about twelve days these reach maturity and the bottle is soon teeming with flies—possibly three or four hundred in number. Many

thousands of flies can therefore be raised in the space available on a laboratory table, and many generations can be observed in the course of a year—a relatively short time for work in breeding.

Millions of Drosophilas have been closely studied by laboratory workers, and it has been observed that occasionally new mutants arise. The eyes may be changed from red to white, the wings reduced to mere stumps, the body color changed from yellowish gray to black, and so on. These changes are relatively rare; possibly just one fly in a hundred thousand or so will show some conspicuous change such as white eyes instead of red. But the change is hereditary, since it is due to a change in a gene, and from the mutant a new race can be derived with the new trait.

The Relation of Genes to Traits.—In the study of heredity we must clearly distinguish between genes and traits. Genes are at the bottom of development. Traits on the other hand are end products of development, as hair color, eye color, size, shape, and so forth. They require both the proper genes and the proper environment for their development.

There is no one-for-one correspondence between genes and traits —not just one gene for hair color, one for eye color, and so forth. On the contrary, every trait is influenced by numerous genes. In Drosophila, for example, there are at least twenty genes for eye color. This is known from the fact that eye color might be changed by mutations in no less than twenty different genes.

Then on the other hand the influence of a given gene is not necessarily limited to a single trait. In man albinos lack pigment in their eyes, hair, and skin. In addition they are not quite as vigorous as people who have pigment. But the difference between normal people and albinos is due to a mutation in just one gene, and this indicates that one gene can influence several parts of the body. However, a gene does not give rise to several parts of the body, or even one part, all by itself, but only in conjunction with many other genes.

In this connection it should be pointed out that one trait might be part of another trait. The hand, for example, is part of the arm, and any gene which influenced the arm, say, through the control of the blood circulation, might at the same time influence the hand. In this particular case it is obvious why the influence of one gene should ramify into different parts of the body. But a gene often influences several traits which are not so obviously connected. In

these cases the apparent lack of connection is due to our ignorance. If we knew more about development we should undoubtedly find that traits which are apparently distinct are really connected in development. Therefore it is not at all surprising that one gene often influences several apparently distinct traits.

In summary, then, many genes interact in the production of one trait, and one gene (in conjunction with others) often influences many traits.

Gene Substitution.—If we were asked what influence a given gene had, all by itself, on development, we should not be able to answer the question. We should be very much in the same position as if asked what influence a given cogwheel had on the running of a watch. Obviously, a cogwheel all by itself would not do anything. If, however, we damaged the cogwheel, we might prevent the whole watch from running; or if we substituted a cogwheel of different size, we might make the watch run faster. Just so with genes. An albino and a normal person agree in their hereditary make-up in all respects except for the fact that the one has a mutated gene in place of a normal gene, and he therefore fails to develop pigment. But it does not follow that the one normal gene in question is entirely responsible for pigment development. Many other genes are also necessary. The albino gene might be held responsible for the failure of pigment to develop, but of course the other genes concerned with pigment development also are not functioning, so that really pigment fails to develop in an albino because none of the pigment genes are functioning. Often a mutation does not cause complete failure of a trait to develop, but it modifies its development, often in a positive way, as when it causes, let us say, an increase in size. This would be like making a watch go faster by substituting a cogwheel of different size for the ordinary one. The mutant gene by itself is not entirely responsible for the new trait (tallness), since all of the other genes for size are necessary for tallness in addition to the one that mutated. But the *difference* between the normal plant or animal and the taller mutant is due to a *difference* in a corresponding single gene of the two organisms. In other words, the difference between them is due to the *substitution* of a single gene.

Loci and Alleles.—In Drosophila the eye color genes are not all bunched up in one chromosome. On the contrary, they are scattered about quite at random among all the chromosomes. Moreover, an eye color gene might be next to a body color gene in one

chromosome but next to a wing gene in some other chromosome. In brief, there is no correspondence between the relative positions of genes and traits. A gene does, however, occupy a definite position within a chromosome. This is referred to as its *locus*.

Figure 13 is a schematic representation of how the genes for a given trait might be distributed among the chromosomes of an animal or plant. The circles within each chromosome represent the genes, and those that are shaded are supposed to represent the genes for some trait, as eye color in an animal or shape of seed in a plant. The unshaded genes are concerned with other traits. Genes at different loci, whether concerned with the same or with different traits, are usually different from one another.

The fertilized egg contains a *pair* of genes at each locus (Fig. 13). The two members of a given pair are known as *alleles* (pronounced al-leels'

Fig. 13. The random distribution of the genes influencing a given trait. The genes shown in black are supposed to influence the trait. Note that they occupy the same "loci" in chromosomes of paternal and maternal origin.

and meaning "of one another") or sometimes as allelomorphs (an older term). Usually the two members of a given pair of alleles are alike, since a fertilized egg is usually derived from two parents which belong to the same species and which are alike in most respects. But sometimes alleles differ, as when a fertilized egg receives a gene for brown eyes from one parent and a gene for blue eyes from the other parent.

What Is Inherited.—We sometimes hear a person state that he inherited his brown eyes from one of his parents. Strictly speaking, this statement is incorrect. Brown eyes are not in the fertilized egg. The only things we inherit are genes. Brown eyes and other traits then develop.

The hereditary material is sometimes referred to as the *germ plasm*. In man and other animals, the germ plasm consists exclusively of chromosomes and genes so far as is known. In green plants, bodies known as plastids (occurring in the cytoplasm)

constitute part of the germ plasm, but apart from these, the germ plasm of plants also consists exclusively of chromosomes, so far as is known. We may, then, define heredity as *the transmission of the germ plasm from parents to offspring.*

The Germ Plasm.—It is difficult to imagine just how small a human being is at the beginning of his existence, when he is a fertilized egg. According to an unpublished estimate made by H. J. Muller the entire human race—two billion creatures in all—could at this very earliest stage in their development have been crowded into a two-gallon bottle!

If the egg is remarkable in being much in little, what must we think of the sperm cell, a body that is incomparably smaller and yet contributes just as much to our inheritance as the egg cell. A two-gallon bottle would by no means be necessary to hold all the sperm cells that fertilized the eggs from which the present-day inhabitants of the world developed. According to an estimate by H. J. Muller all these sperm cells—containing the entire paternal inheritance of two billion human beings—all could have been packed into a space about twice the size of a pin head!

The nucleus of an egg is not as compact as that of a sperm cell mainly because it contains a relatively large amount of water. But if we could remove the water from the egg nucleus, then the maternal inheritance of the entire human race could also have been packed into a space about twice the size of a pin head. It is this remarkable material—the germ plasm—that we are about to study. We might in fact define *genetics* as *the science which concerns itself with the study of the germ plasm*—with its origin, its constitution, its alterations, its transmission to the offspring, its interaction with the environment, and its influence on development.

SUMMARY

1. Chromosomes constitute the physical basis of heredity. They pass from one generation to the next through the reproductive cells, and in some manner not fully understood they control development and cause the offspring to resemble their parents, in a given environment.

2. Chromosomes contain the genes, the ultimate units of heredity. Genes are arranged in linear order within the chromosome, like the beads on a string of beads.

3. Parents transmit equal numbers of chromosomes to their offspring. They therefore have an equal influence in heredity.

4. There is only one chromosome of each kind in a sperm or egg cell. In the fertilized egg there are two of each kind. The sperm and egg cells are said to be *haploid*, the fertilized egg *diploid*. The total haploid complement of chromosomes is referred to as a *set*.

5. When the fertilized egg prepares to divide, each chromosome first splits into two. Each new cell then receives one of the split halves—a "sample" of each chromosome—and it therefore has exactly the same number and kinds of chromosomes as the fertilized egg. Cell division preceded by chromosome division is referred to as *mitosis*.

6. Most of the cells of the body are derived from the fertilized egg by mitotic cell division. Hence all such cells are chromosomal *duplicates* of the fertilized egg.

7. The sperm and egg cells contain half the number of chromosomes found in ordinary cells, and they arise through a special kind of cell division referred to as the *reduction* division, at which the members of each pair of chromosomes first come together in the middle of the cell and then separate to opposite poles. Each daughter cell therefore gets only one member of each pair, and as a result the sperm and egg cells are haploid.

8. Chromosomes and genes as a rule maintain their identity from one cell division to the next and from one generation to the next, and for this reason a given form of life maintains its identity.

9. Sometimes a gene undergoes a permanent change known as a *mutation*. This might result in a new or *mutant* race, such as a white-flowered variety of peas instead of the normal red.

10. Many genes interact in the production of any trait (such as red flowers), but a mutation in just one gene might cause a change in a trait (as when red flower color changes to white by mutation).

11. The influence of a given gene (in company with others) is not restricted to a single trait. This is shown by the fact that a mutation might influence two or more traits.

12. The genes for a given trait are scattered quite at random throughout all the chromosomes, but each kind of gene occupies a definite position in a chromosome, referred to as its *locus*.

13. At each locus there are a pair of genes in a given pair of chromosomes. Genes which occupy the same locus are said to be *alleles*.

14. The hereditary material is collectively referred to as the *germ plasm*. In animals the germ plasm consists exclusively of chromosomes, so far as is known. In green plants, bodies known as plastids (occurring in the cytoplasm) constitute part of the germ plasm, but apart from these the germ plasm in plants also consists exclusively of chromosomes, so far as is known.

15. *Heredity* is the transmission of the germ plasm from parents to offspring.

16. *Genetics* is the science which is concerned with the study of the germ plasm.

PROBLEMS

1. If hereditary bodies were transmitted through the cytoplasm (rather than through the nucleus), then would a child more often resemble his mother or his father? Why?

2. How can we most readily account for the fact that a child resembles both parents equally?

3. According to Galton's *Law of Ancestral Inheritance*, a person receives half his hereditary qualities from each parent, one-quarter (on the average) from each grandparent, and so on. Account for this law in terms of the physical basis of inheritance.

4. Assume that it were possible successfully to transplant the fertilized egg from the uterus of a white woman to that of a colored woman, in equally good health as the white woman and living under equally good circumstances. Do you suppose the resulting newborn child would be any different in his physical or mental make-up from what he would have been if he had developed in his mother? Give the reason for your answer.

5. It is possible at present to rear a six months' child in an incubator (with a small per cent of successes). Suppose that with further improvements in technique it would be possible some day to get a five months' child to live and then one of four months, three months, etc. Would the hereditary qualities of the child be altered? At what moment are the hereditary qualities of the child determined?

6. Are blue eyes present in the fertilized egg? Strictly speaking, then, would you say that blue eyes themselves were inherited? What, in connection with blue eyes, is inherited? What else is necessary for the development of blue eyes?

7. In general, would you say that traits such as blue eyes were inherited or that they develop? What two factors are necessary for the development of traits in general?

8. It is sometimes stated that heredity is the resemblance between individuals related by descent. Would you say that such resemblance was itself heredity, or an expression of heredity? How would you define heredity?

9. Might heredity be responsible for some of the differences between blood relatives? Give an example in illustration of your answer, involving eye color differences between children in the same family.

10. If two people had exactly the same heredity and the same environment, would they merely tend to be alike or would they actually be alike?
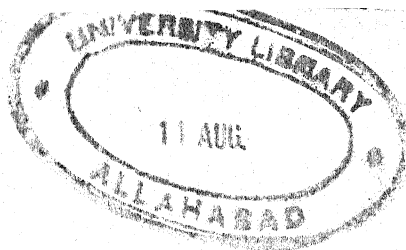
2. HEREDITY AND ENVIRONMENT

HEREDITY is important in determining what we are, but we must not overlook the environment—the air about us, warmth and cold, and numerous other agents with which the body comes into contact. Human beings themselves are part of our environment, and they influence us through speech and in other ways. Thus such intangible things as education are part of our environment. Even one part of us may furnish the environment for another part, as when the thyroid gland produces a secretion which whips up oxidations in other parts of the body. Getting down to the cell, we find that cytoplasm surrounds the nucleus and is part of the environment of the nucleus. But even within the nucleus one chromosome is surrounded by others, and one gene by other genes. Thus everything outside of a given gene constitutes the environment of that gene.

A human being or any other creature is the product of both heredity and environment, since each plays a part in his development. The importance of heredity has already been stressed. But the environment is also important in development, as can be readily seen when we compare a child who has been well nourished with one who has not been. Sometimes a very special environment is necessary for the development of a trait. For example, in Drosophila there is a race which develops the normal three pairs of legs when grown at room temperature but which in the cold develops six pairs (by the splitting of each leg). In the breed of rabbits known as Himalayans, warmth causes the fur to become white; coolness causes it to become dark. Himalayans are white except for the tips of the feet, tail and ears. These parts are dark because they are not quite so warm as the rest of the animal. Plucked-out white fur is replaced by black if the rabbit is kept cool while the new fur is growing. Common experience as well as controlled observations in

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the laboratory furnish almost innumerable examples of the fact that the environment influences development.

The Relative Importance of Heredity and Environment.— If we knew that a fertilized egg had the genes for blue eyes, we could be reasonably sure that the egg would develop into a blue-eyed person, not brown. Had the genes been for some mental trait, say, mathematical ability, then we could not be so sure as to what would happen. We should have to consider the special environment in which the person develops, because anyone might appear very stupid at mathematics if he had the wrong training along these lines even though he had the right heredity. In this respect the mental trait in question is different from a physical trait such as blue eyes.

But we could not reasonably conclude on this account that blue eye color was entirely a matter of heredity and mathematical ability entirely a matter of environment. Each trait requires both heredity and environment for its development. It so happens, however, that the normal environment supplies all the conditions necessary for blue eyes, possibly because this trait develops in large measure while the embryo is in the uterus of the mother where the relatively few conditions necessary for its development are sure to be found. The conditions that influence the development of a mental trait are on the other hand extremely numerous. They come into play during the entire life of a person and appear in the most diversified forms in his environment. They would not be likely to be the same for any two persons. As a result a mental trait shows a good deal of variation.

The question "Which is more important, heredity or environment?" raises a problem which really has no answer. It puts us in the same sort of difficulty as if someone wanted to know whether a fish's tail or the water in which he swims is more important for his swimming. Obviously both are absolutely necessary and neither can be said to be more important than the other. So with heredity and environment. The fertilized egg could not develop without its chromosomes; neither could it develop if it were removed from the womb of the mother and deprived of proper nourishment, warmth and other environmental conditions. In particular, the fertilized egg could not develop into a being with human intelligence unless it had both the genes for human intelligence and also the proper environment.

Hereditary and Environmental Differences.—Two people might have the same genes for mental ability and one might be more intelligent than the other because of a difference in their environments. In this case the *difference* in their intelligence was due to the environment. But each person's intelligence when considered by itself was due *both* to his heredity and the environment in which he developed. Usually when the question is asked as to whether a certain thing is due to heredity or environment, what we have in mind is some *difference*. When, for example, we ask whether genius is due to heredity or environment what we want to know is whether the *difference* between a genius and a normal person is due to the one or the other.

Often it is difficult to tell whether a special mental trait is due to heredity or environment. The children of a bank president may follow in the footsteps of their father largely because of their family connections and not necessarily because of special kinds of genes that make for executive ability. Even temperamental differences such as we notice between different races or peoples may be due partly to the environment; witness the speed with which foreigners become Americanized and lose their marked temperamental peculiarities once they are no longer in their old social environment. We must, therefore, be very cautious in referring special mental traits entirely to heredity. At the same time it would be very odd if such a complicated organ as the brain were exactly alike in everybody by heredity. Probably every organ of the body, the brain included, is subject to hereditary variations.

Very often variations in a trait, whether mental or physical, are due to either heredity or environment or both. We might take size in illustration. Two children in the same family might differ markedly in size at a given age. Yet they might both have been equally well nourished. The difference in their size is due to a difference in heredity often found between members of the same family. On the other hand we know that differences in nourishment might cause two children to differ in size. In this case the difference is due to the environment. It is also possible that one child might be taller than another by heredity, and that he might be better nourished in addition. In this case the difference in the size of the two children is due to a combination of heredity and environment. In like manner the mental differences between people might often be due to either heredity or environment or a combination of the two.

In order to determine the influence of heredity alone, we must make the environment the same, for we then rule out the environment as the cause of any difference. In like manner, in order to determine the influence of the environment alone, we must make
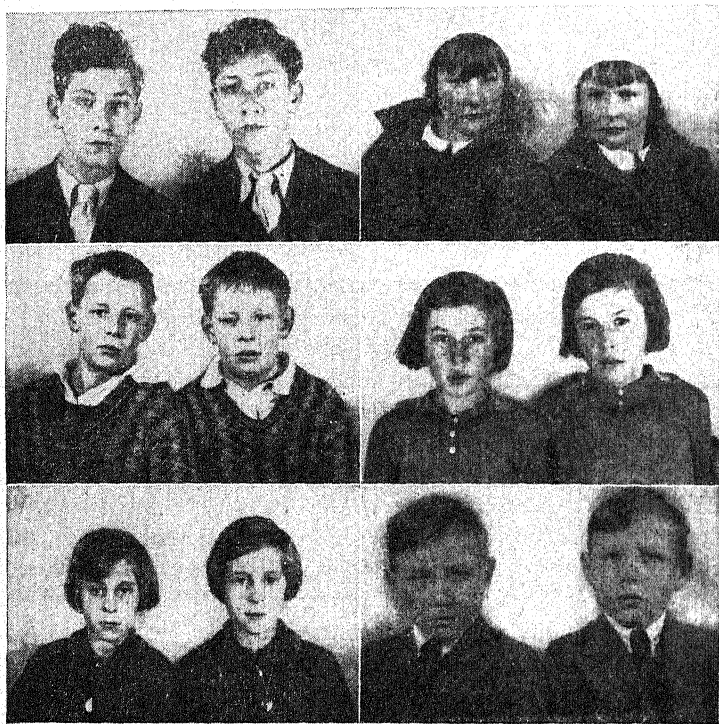
Fig. 14.1. Six pairs of identical twins. In all these pairs both members belonged to the same blood group and had almost exactly the same shade of eye color and hair color. Both of the girls of the lower left pair had only two upper incisor teeth. In both boys in the lower right figure the second and third toes of both feet are joined by a fleshy web. (From photographs by Dr. R. Stohler, in Holmes' *Human Genetics and Its Social Import*, Courtesy of The McGraw-Hill Book Co.)

heredity the same, for we then rule out heredity as the cause of any difference.

Twins.—Twins are of great value in the study of heredity and environment. But first a word about the nature of twins. Twins are of two kinds: those that are no more alike than ordinary brothers and sisters (except that they are of the same age) and those that are

so much alike that it is often difficult to distinguish them apart. The first are known as *fraternal* twins, the second as *identical* twins. About one birth in eighty is a twin birth in the population as a

FIG. 14.2. Identical twins studied for likeness. According to a "diagnostic" formula of D. C. Rife, there is only one chance in 480,000 that the upper left pair of twins are not identical. This estimate is based on a study of their detailed resemblance (in regard to blood groups, eye color, finger prints, intelligence, etc.). In the case of the upper right pair, the chances are 1 in 320,000; lower left pair, 1 in 5,000. (From Rife in *The Journal of Heredity*.)

whole. This figure includes both kinds of twin births (fraternal and identical). Identical twin births are almost one-fourth as frequent as fraternal twin births. Therefore about one birth in three hundred twenty is an identical twin birth.

Fraternal twins develop from two separate eggs, each fertilized

by a separate sperm cell. This accounts for the fact that fraternal twins are no more alike than non-twin children in the same family, except that they are of the same age. Identical twins on the other hand develop from a single egg which in the course of development becomes two by the division of the developing egg into two separate embryos. The division of the original embryo is usually down the middle of the body, so that the left half of the embryo becomes one twin, the right half the other one. In very exceptional instances the egg, by further division, gives rise to identical triplets, quadruplets, or quintuplets. From the fact that identical twins develop from the same fertilized egg, they are often referred to as *mono-zygotic* twins (one-yoked). They are also referred to as *mon-oval* twins (one-egg).

The Detailed Resemblance of Identical Twins.—Identical twins who are reared together have very much the same environment, as well as the same heredity, and they show us the remarkable extent to which two human beings can resemble each other when both heredity and environment are alike. Figs. 14.1 and 14.2 are photographs of identical twin couples. The resemblance between the two members of a given twin pair applies not only to their facial features but also to their general physical build, their deportment, their facial expression, and often to such slight details as the manner in which they hold their head upon being photographed.

The detailed resemblance of identical twins is often strikingly shown by a comparison of their finger prints. No two finger prints are ever exactly alike, not even those of corresponding fingers of left and right hands of the same person. But the finger prints of left and right hands are often very similar in their *general pattern*, though they may also be very different. The reader can perhaps verify these statements by comparing the finger prints on corresponding fingers of his own left and right hands. Now the finger prints of identical twins are often very similar in their general pattern, but they also often are markedly different (Fig. 15). In general it may be stated that the finger prints of identical twins are about as much alike on the average as are those of the left and right hands of the same person.

It should be borne in mind that the left hand of one identical twin and the right hand of the other would have been left and right hands of the same person had the developing egg not divided into

two. It is therefore not surprising that the finger prints of identical twins should show on the average the same degree of resemblance as finger prints of opposite hands of the same person.
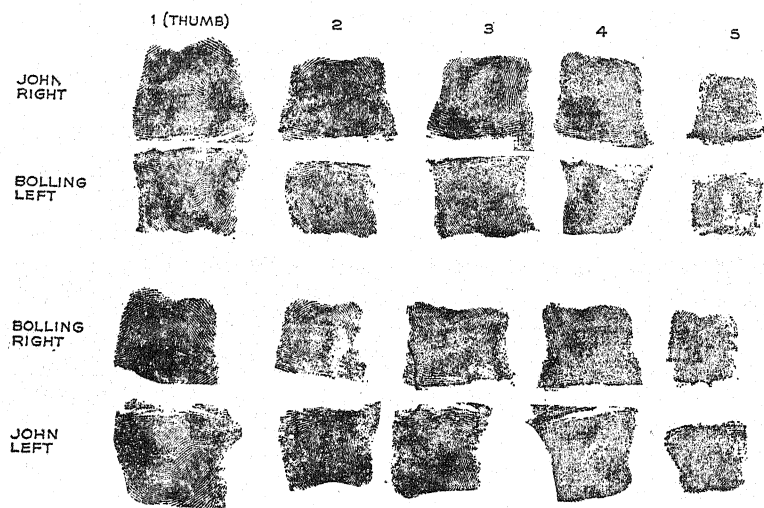
FIG. 15. The fingerprints of identical twins. Note that the two upper left fingerprints (thumbs) are mirror images; also, that John's right thumb is more like Bolling's right than his own left. In the case of the second (index) finger, note the close similarity in all four fingers and the mirror imaging. Note the same for the third fingers.

In general the left and right sides of a person's body are mirror images of each other. This applies to the hands as a whole, and it often applies to the finger prints, though not always. In the case of identical twins the finger prints of the left hand of one twin are sometimes mirror images of those of the right hand of the other, though sometimes finger prints of corresponding hands are mirror images. Pattern resemblances and mirror imaging are often strikingly shown by the whorls of hair on the crown of the head in the case of identical twins (Fig. 16).
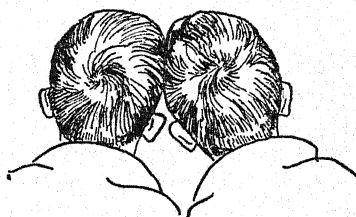
FIG. 16. Symmetry reversal (mirror imaging) in identical twins. (From C. E. Lauterbach in *Genetics*.)

There is somewhat of a tendency for identical twins to be opposite-handed (one right-handed, the other left-handed). This tend-

ency does not by any means express itself in every twin couple, but identical twins are more often opposite handed than are fraternal twins. Moreover, the proportion of left-handed people found in identical twin pairs is definitely greater than in the population as a whole. One theory of left-handedness is that left-handed people are always or at least usually members of an identical twin pair, the other member often having died before birth. However, this theory, though possibly true, has little evidence in its support.

Identical twins are not only alike physically but also mentally. Intelligence tests show that they are, on the average, much more alike in mental aptitude than are fraternal twins. The general responses of identical twins are also very much alike. If one is slow, the other is also likely to be; or if one is nervous and excitable, the other is also likely to be.

Handwriting can be regarded as a response, and this in the case of identical twins shows a resemblance, particularly when the twins are reared together (Fig. 17). But it should be emphasized that heredity would simply make the resemblance *general*, not detailed.

a. TWIN PAIR A.

b. TWIN PAIR B.

FIG. 17. The handwriting of identical twins reared together.

Thus, for example, if one twin were nervous and wrote with a shaky hand, the other would also be likely to; or if one wrote a heavy clumsy hand, the other would also tend to do likewise. But heredity would not cause one twin to loop and curve his *l* in exactly the same way as the other one. Any such detailed resemblance would be due to the added influence of similar training. The two twins might, for example, have had the same teacher, might have been taught at the same time, might have copied from the same writing, and so forth.

They would also tend to imitate one another more than would fraternal twins because of the closer association usual between identical twins. All this would represent environment. The handwriting of identical twins who were reared apart from early infancy does not show the same degree of resemblance as that of twins who were reared together (Fig. 18).

FIG. 18. The handwriting of identical twins reared apart. (Traced from a photograph in Newman, Freeman and Holzinger, *Twins*, permission of The University of Chicago Press.)

Intelligence Tests on Identical Twins Reared Apart.—In order to determine the influence of the environment on mental ability, we might compare identical twins who have been *reared apart*. Any difference between the twins must be due to environment, since heredity is the same and has been ruled out as the cause of the difference.

Intelligence tests were made by Muller on a pair of identical twins reared apart from early childhood, followed by tests on nineteen pairs made by Newman, Freeman, and Holzinger. The members of each twin pair as a rule rated very closely together in the tests, much more closely than would be expected as a mere matter of chance, and apparently the difference in their environments caused very little difference in their mental development. But in most cases the environment of the twins was not so very different, despite the fact that the twins were reared apart. They were reared in the same *social* environment, and so they had substantially the same opportunities for mental development. But in two cases there was a greater difference in the environments, and in these cases the ratings of the twins showed a corresponding difference. In one of the two pairs there was a difference of 30 points in the intelligence tests or as great a difference as that which separates a normal person from one somewhat subnormal. It would be interesting to see the results of intelligence tests made on identical twins if one member of the pair were adopted by a college professor and his wife and the other by a couple in an isolated mountain community. No tests of this sort have as yet been made.

The Method of Twin Comparison in the Study of Human Heredity.—It is obvious from what has just been said that twins are of great value in the study of heredity and environment. We might give a further example of how twins might be of value.

Are all people equally susceptible to .tuberculosis, or are some more susceptible than others, by heredity? In order to answer this question it would not be sufficient simply to see whether tuberculosis runs in certain families, for it might well happen that infected mothers transmit the disease to their children, and that the disease might thus persist for many generations in a given pedigree, apart from any special hereditary susceptibility to the disease. But twins can help us in our problem. Suppose we should select a fairly large number of tubercular people who were members of a twin pair and we should find that the other member of the pair was more often also tubercular in the case of identical twins than in the case of fraternal twins. We could then conclude that susceptibility to tuberculosis was at least in part hereditary, since fraternal twins are reared together just as often as identical twins, and so the environment has been ruled out as the cause of the difference in the frequency of joint susceptibility in the two cases. Studies were made by Kallmann and Reisner of 308 persons who were members of twin couples. In the case of identical twin couples it was found that when one twin had tuberculosis, the other also had it in 87.3 per cent of the couples studied, but for non-identical twins the figure was 25.6. This shows that susceptibility to tuberculosis has an hereditary basis, though of course the environment plays an important part in the spreading of the infection.

The method of twin comparison is now coming into extensive use in the study of human heredity.

Hereditary Genius.—We might now say a word or two on the question of hereditary genius.

In the family of Johann Sebastian Bach musical ability can be followed for five successive generations (Fig. 19). Johann Sebastian Bach himself had twenty children (seven by his first wife and thirteen by his second), and among these children there were five outstanding musicians. Bach's first wife (Maria Barbara) was an accomplished musician. She was his cousin and was the daughter of a Bach, himself a musician. Bach's second wife was also highly talented, though no close relative of Bach. There was an interesting case of twins in the Bach family tree (indicated by two lines that

come off at the same point in Fig. 19). Bach's father, Johann Abrosius, had a twin brother, Johann Christoph, who was so like him that (to quote Schweitzer) "even their respective wives could only distinguish them by their clothes. Speech, sentiments, the
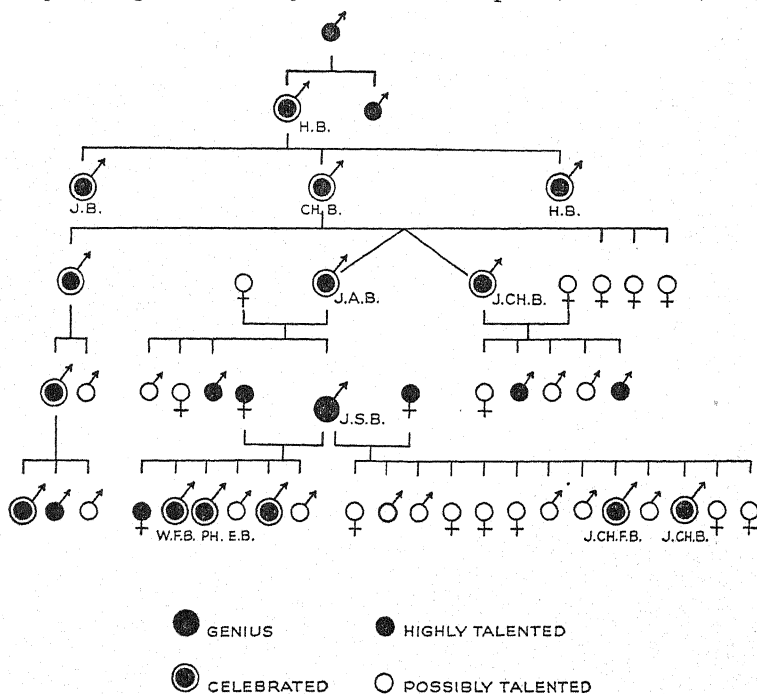
Fig. 19. Musical genius in the Bach family. The large black circle labelled J.S.B. represents John Sebastian Bach. J.A.B. and J.Ch.B. were identical twins. (From Baur, Fischer, Lenz, *Human Heredity*, by permission of The Macmillan Company, publishers.)

style of their music, their methods of performance—all were alike. When one was ill, so was the other. They died within a short time of each other."

Musical talent can also be followed through several generations of the Mozart family. Mozart himself composed sonatas and performed before the crowned heads of Europe at the age of five. It is of course true that in both the Bach and Mozart families the children were reared in a musical environment, and this undoubtedly contributed to their genius; but it seems unlikely that environment could have accounted entirely for their genius.

Galton studied the family trees of 415 celebrated Englishmen. He found that the fathers and sons and other close relatives of these men were outstanding in an unusually high proportion of the cases. Galton concluded on the basis of his studies that exceptional ability was hereditary. But in England the social caste system is highly developed, and privileges are limited to certain favored groups who comprise relatively few families (compared to the total in England). It is therefore not surprising that success is largely limited to these families. Perhaps part of their success is due to special heredity, but undoubtedly some part is due to their special environment. It seems likely that a person reared in the slum section of London would seldom if ever become outstanding for high achievement even if he had an identical twin brother who had been reared in a favored family and had become distinguished.

Galton also found that noted military leaders such as Alexander the Great and Hannibal belonged to families which produced other noted military leaders. But in many countries the army leadership is recruited from certain families in which the military tradition is passed from father to son. Here again the environment might be partly responsible for success.

Sometimes successful people owe their success to their ability to exploit the talents of other people. According to some commentators Napoleon owed no small measure of his success to his marshals. It is often difficult to tell whether or not we are really dealing with genius. What is considered genius in one age or by one set of standards might not be considered so in another age or by another set of standards.

We might summarize by saying that every case of genius must be separately studied before we can determine the relative influence of heredity and environment in shaping it.

Heredity and Environment Extremists.—Some people say that heredity is everything. These are the "heredity extremists." They claim that all differences in mental ability are entirely hereditary. Obviously they ignore the environment, particularly the social environment. This is bound to have a great influence on a person's mental development.

An example often cited by the heredity extremist is the Jukes case. Jukes was a backwoodsman in western New York, described as shiftless and good for nothing. In the course of eight generations he had over two thousand descendants, many of whom were

drunkards, prostitutes, and dependents on public charity. All this degeneracy has been ascribed to bad heredity. However, the Jukes case is obviously complicated by the effects of the environment. For the children of drunkards and prostitutes would not have the best sort of home surroundings, and this in itself might account for at least part of the degeneracy observed. Environments can persist from one generation to the next, and they can imitate heredity in the persistence of their influence. Perhaps the degeneracy of Jukes does represent a case of bad heredity. But we do not have proof that it is to be explained entirely in this way. The heredity extremists have been ignoring the social environment in their interpretation of this case. The Jukes were perhaps in the same class as the "Tobacco Roaders," whose "degeneracy" seems at least in part to have been associated with an economic depression in their section of the country, involving them in particular. They were previously normal American stock, so far as we know.

On the other hand there are those who say that environment is everything. These are the "environment extremists." They do not believe in heredity at all. They say that mental development is entirely a matter of environment—getting a good start and coming under the right influences. This viewpoint implies that everybody is born with exactly the same inherent ability and that all later differences are due entirely to environment. The contention of the environment extremists seems unlikely in view of the differences in intelligence shown by children in the same family, or of the greater similarity in the intelligence of identical twins as compared to fraternal twins.

The Value of Physical Traits in the Study of Heredity.— The student of heredity must as a rule deal with traits that are rather fixed in their development in order that he might not be confused by the effect of environment. He wants to know what kind of genes parents transmit to their offspring and how they transmit them. The study of these processes, in fact, constitutes the science of heredity. But genes are ordinarily hidden from view and they manifest themselves to us only through the influence they have on development. It is therefore necessary in heredity to work with definite physical traits. Nevertheless, once we understand the hereditary basis of physical traits we are prepared to go to mental traits and understand them—perhaps even control them.

SUMMARY

1. Heredity and environment are equally important in the development of the individual.

2. Some traits (such as eye color) depend on environmental conditions which do not vary much from one individual to another. All individuals having the same kind of genes for such a trait are therefore relatively uniform with regard to that trait. Other traits, including in particular intelligence, depend on environmental conditions which vary enormously from one individual to another. Hence individuals genetically alike with regard to intelligence might differ greatly.

3. A *difference* between two individuals might be due exclusively to a *difference* in either their heredity or their environment. Often it is due to a difference in both.

4. If two individuals have the same heredity (as identical twins), then any differences between them must be due to a difference in their environments; *vice versa*, if they have exactly the same kind of environment (a condition difficult of achievement), then any differences between them must be due to heredity.

5. Twins are of great value in studying the relative influence of heredity and environment in causing differences between people. Studies have been made on identical twins who have been reared apart in order to see to what extent they differ in their intelligence and emotional responses as a result of their different environments. Such studies indicate that environment alone causes less difference than environment plus heredity but that it nevertheless does cause some difference, and this may be rather large when the environments are sufficiently different.

6. Some studies indicate that when one identical twin has tuberculosis the other also has it in about 85 per cent of the twin-couples studied, but in the case of non-identical twins, the figure is only 25 per cent. This indicates that differences between people in their susceptibility to tuberculosis has an hereditary basis. In general, in order to determine whether differences in a trait have an hereditary basis in man, we might compare the relative frequencies with which identical and non-identical twins have the trait in common, in twin pairs in which the trait occurs (in at least one twin).

7. It is often difficult to disentangle the relative parts played by heredity and environment in shaping the differences between people. The social environment is especially important in the development of intelligence and other mental traits.

PROBLEMS

1. Suppose that A belonged to the lower social class (with few opportunities for education) and B to the middle social class. Do you think that as a rule A would rate as highly on an intelligence test as B? Might A nevertheless have equally good genes for intelligence as B?

2. In rating the comparative intelligence of people by means of intelligence tests, what precaution is necessary as regards the social status of the people compared?

3. Often the finger prints of corresponding fingers (say, the left and right index fingers) of a given person show symmetry reversal (one finger print is the mirror image of the other). In identical twinning the developing embryo divides down the middle, the left half giving rise to one twin, the right half to the other. Given the left index finger of one identical twin. Which index finger of the other twin would you expect more often to show symmetry reversal, left or right (assuming that one of them showed it)? Why?

4. According to Watson (an American psychologist of the behaviorist school), a child is born with only three "unconditioned" reflexes or responses (sucking, fright at falling, and fright at a loud noise), and all other responses are "conditioned" reflexes and dependent on experience (as when a large ferocious animal becomes associated with a loud noise and thereby becomes the stimulus for fright). Watson therefore concludes that all people are alike at birth (by heredity) and that all later differences are solely the result of experience (environment). Might Watson's premise be true, without necessarily his conclusion? Tell why. (*Hint:* Would two people necessarily be equally susceptible to such conditioning stimuli as those involved in musical training, mathematical training, etc. Why?)

5. Certain people (known as carriers) are immune to typhoid in the sense that they can harbor large numbers of typhoid germs in their intestines without apparent injury to themselves.

a. Suppose all people were equally exposed to typhoid germs (all were infected with the germ) but that only a small per cent developed typhoid. To what would the disease usually be attributed, hereditary susceptibility or environment (the germ)? Would it nevertheless be due to both? Why?

b. Suppose nobody were immune to typhoid (if a person had the germs he would have typhoid) but that only a small per cent of the population were exposed to typhoid germs. To what would the disease now be attributed, heredity or environment? Would it nevertheless be due to both? If so, what is the hereditary factor involved? the environmental factor?

3. MENDEL'S PRINCIPLE

THE MODERN science of heredity starts with the experiments of the Austrian monk Mendel on pea plants (Fig. 20). Mendel published his results in 1866 but he received very little attention and his work was overlooked for a number of years. In 1900 the principles of heredity which Mendel had discovered were independently rediscovered by three biologists: de Vries, Correns, and von Tschermak. From that time on the science of heredity has made rapid progress.

Mendel's Experiments.— Mendel crossed various races of edible peas (*Pisum sativum*). For example, he crossed a red-flowered race with a white-flowered race. He did this by dusting the pollen of the one race, say, the white, on the pistils of the other (the red). Of course he prevented the red plant from pollinating itself. This he did by removing the stamens of the red flowers before the flowers had opened and shed their pollen. After pollinating the emasculated red flowers with the white pollen, he enclosed them snugly in bags in order to prevent insects from getting to them with pollen from unknown sources. Thus he "crossed" the red race with the white. The offspring of the cross were red. Mendel then self-fertilized the offspring and

FIG. 20. Johann Gregor Mendel. (From the Mendel Museum in Fredericksburg, Va., Courtesy Dr. H. Iltis).

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he found that they produced offspring of their own in the ratio of 3 reds : 1 white.

We can most easily understand Mendel's results in terms of chromosomes and genes. Red is the normal flower color of peas and
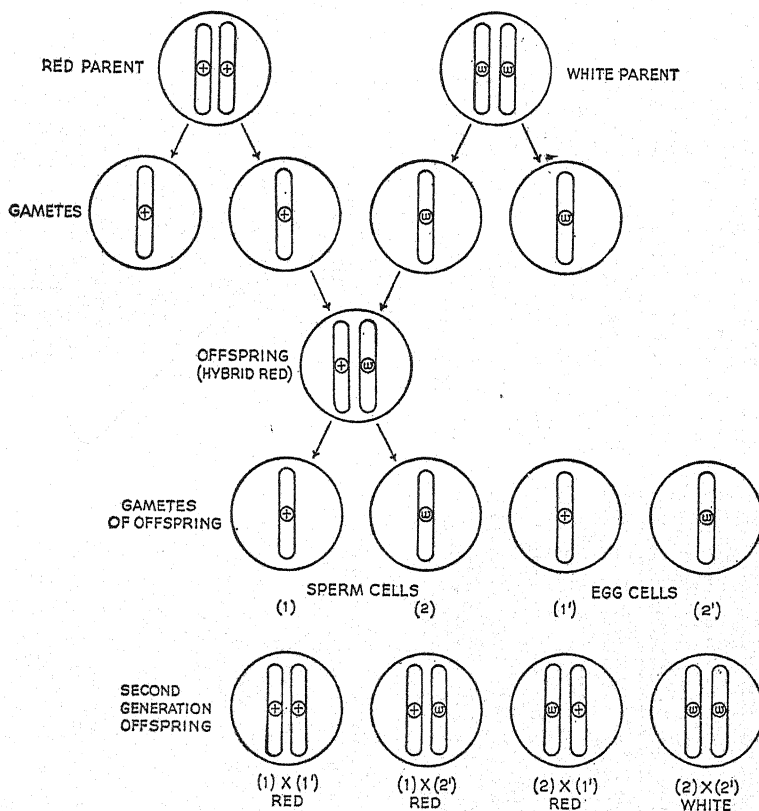
FIG. 21. The cross of a red-flowered race of peas by a white-flowered race.

is due to the interaction of many normal genes. A mutation took place in one of the normal genes and caused the color to change to white. We can label the normal gene in question + and the mutant gene *w* (white), as shown in Fig. 21. In this figure only one pair of chromosomes is shown, though there is more than one pair in peas; only the genes with which we are concerned are shown. Both + and *w* occupy the same locus and are referred to as *alleles*. The gene + is said to be the normal allele of *w*. It would not be

strictly correct to refer to $+$ as *the* gene for red flowers as this
would imply that $+$ was the only gene for flower color; hence we
refer to $+$ as the *normal allele* of white. We shall refer to the locus
occupied by either $+$ or w as the white locus (after the mutant
gene).

A pure red plant has a pair of $+$'s at the white locus, and its
formula is $+/+$. In technical terms it is said to be *homozygous* (or
pure) for $+$. All of its gametes (or sperm and egg cells) are $+$. A
white plant has a pair of w's at the white locus (is homozygous for
w), and its formula is w/w. All of its gametes are w. (In flowering
plants the sperm cells as well as the eggs are spherical in shape and
both are represented as circles in Fig. 21.) When the red and white
plants are crossed, the $+$ and w gametes combine and produce off-
spring of composition $w/+$ (Fig. 21, middle). These offspring
carry a pair of unlike alleles at the w locus and are therefore said
to be *hybrid* or *heterozygous* at the white locus. They are red in
spite of the fact that they carry w as well as $+$. The normal allele
($+$) is said to be *dominant* and white is said to be *recessive*.

In the hybrid, $+$ does not mix with w, and when the reduction
division takes place $+$ and w separate from each other (Fig. 21).
As a result, half the gametes get $+$ and half get w. When the
hybrids are self-fertilized the gametes come together in the com-
binations shown in Fig. 21, lower part. A $+$ sperm cell (1) might
fertilize either a $+$ or a w egg; so might a w sperm cell (2). The
hybrids therefore produce offspring in the ratio of $1 +/+ : 2$
$w/+ : 1\ w/w$. The first two classes appear red and the third class
white. Hence the hybrids produce offspring in the ratio of 3 red : 1
white. In Mendel's experiment the actual count of the offspring was
705 red and 224 white, or 3.01 red to .97 white. This is a close
approximation to the ideal 3 : 1 ratio.

In the above cross the original red and the white races constitute
the first parental generation and they are usually designated by the
symbol P_1. The hybrids produced by the cross constitute the first
filial generation, usually designated by the symbol F_1. The off-
spring of the hybrids constitute the second filial generation, or F_2.

In summary then, when a red race of peas is crossed to a white,
the P_1, F_1, and F_2 are as follows:

P_1 red $(+/+)$ $\times$ white (w/w)
F_1 red $(w/+)$
F_2 $1 +/+ : 2\ w/+ : 1\ w/w$, or 3 red : 1 white

Mendel could not tell by direct observation that some of the F_2 reds were pure $(+/+)$ and others hybrid $(w/+)$. But he self-fertilized the F_2 plants and he found that one red in three threw nothing but red offspring and therefore was $+/+$ and that two in three threw both red and white and therefore were $w/+$. The whites produced only whites and must have been pure.

Mendel made a very important discovery when he found that the F_1 hybrids threw pure reds and pure whites, for it had been previously believed that "red" and "white" would mix in the hybrid and that the hybrids would produce only intermediates. But Mendel found that white and its normal allele did not mix in the hybrid, for otherwise the hybrid could not have produced pure reds and pure whites.

We might define Mendel's principle as the *non-mixing of alleles* in the hybrid. This is the most fundamental principle in all heredity.

The 1 : 1 Gametic Ratio.—Mendel did not know in advance of his experimental results that the F_1 hybrids produce the two classes of gametes ($+$ and w) in equal proportions. This he could conclude only from the results that he got in the F_2. He had first to show that in the F_2 the offspring were produced in the ratio of $1 +/+ : 2 w/+ : 1 w/w$. Mendel then figured out that this ratio could be got only if the F_1 hybrids produced two classes of gametes ($+$ and w) in equal numbers.

Another way of proving the same thing is to breed the F_1 hybrid reds to a white ($w/+ \times w/w$). The offspring produced by this cross are of two classes: half are red and half are white, and by self-fertilizing them it can be shown that they are $1 w/+ : 1 w/w$. Since the white parent contributes only w to the offspring, the hybrid red parent must have contributed the $+$ to the $w/+$ offspring, and also one of the w's to the w/w offspring. Since the $w/+$ and w/w offspring are produced in equal numbers, the hybrid red parent must have produced $+$ and w in equal numbers.

The above cross ($w/+ \times w/w$) is sometimes referred to as a *back cross* (because the hybrid is being crossed back to the recessive parent). It is also referred to as a *test cross* because it is a direct test of the gametes of the hybrid.

Genotype and Phenotype.—In the cross of red by white we can classify the F_2 plants either according to their genetic composition ($1 +/+ : 2 w/+ : 1 w/w$); or we can classify them accord-

ing to their appearance (3 red : 1 white). A class as determined by genetic composition (a genetic class) is sometimes referred to as a *genotype;* one determined by its appearance is referred to as a *phenotype.* The $+/+$ offspring constitute a genotype or genotypic class; the reds constitute a phenotype or phenotypic class. A red plant might belong to either one of two genotypes ($+/+$ or $w/+$). By the term *genotypic ratio* we mean a ratio based on genotypes (as $1 +/+ : 2\ w/+ : 1\ w/w$). By *phenotypic ratio* we mean one based on phenotypes (3 reds : 1 white).

Chance Departures from the 3 : 1 Ratio.—If Mendel had grown just four plants in the F_2, he would not necessarily have got 3 reds and 1 white. The 3 : 1 ratio is an *average* result. A similar sort of thing applies to the tossing of a coin. On the average we expect heads and tails equally often, and we can express this fact by the statement that if we toss a coin we get heads and tails in the ratio of 1 head : 1 tail on the average. However, if we toss a coin just twice, we might get a run of two heads or two tails. If the number of tosses is greater, say, one hundred, we should very seldom get a run of all heads or all tails, but as a rule we should get a closer approximation to fifty heads and fifty tails (or a 1 : 1 ratio). In general, the larger the number of tosses the closer would be the approximation to the 1 : 1 ratio, because the disturbing effect of runs would be less.

In the case of the breeding of the two hybrids above considered the sperm and egg cells undergo four possible combinations, and on the average these four tend to occur with equal frequency. But if we are considering a small number of combinations we might get runs of one or another of these combinations and hence of one or another kind of offspring. The larger the number of offspring the closer will be the approximation to the 3 : 1 ratio.

Further Experiments Demonstrating Mendel's Principle.—Mendel did not confine his experiments to crosses between races of different flower color. He also crossed a tall race (which climbs) to a dwarf race. The F_1 were tall and the F_1 when "self-fertilized" gave F_2 offspring in approximately the ratio of 3 talls : 1 dwarf. The actual F_2 count was 787 tall and 277 dwarf, or 2.96 tall : 1.04 dwarf. This is approximately 3 : 1.

Especially favorable material for Mendel's experiments were races that differed in regard to seed characters. A seed can be regarded as an immature plant, and if the seeds have distinctive

traits based on heredity, the labor and space involved in growing them to maturity is saved. Working with seed characters is especially advantageous in the F_2, when large numbers of individuals are required to establish the 3 : 1 ratio.

Mendel crossed a yellow by a green seeded race. Yellow is probably the normal race and green then arose from yellow by a mutation. We can indicate the mutated gene by the symbol g and its normal allele by $+$ (the gene from which g arose in some yellow ancestor of the green race). It is to be understood that the $+$ symbol in the present experiment stands for a different gene from the one indicated by a $+$ in the previous experiment. The parents then were $+/+$ (yellow) $\times$ g/g (green). They produced F_1 seeds of genotype $g/+$. In color the F_1 were yellow ($+$ being dominant to g). The F_1 seeds were planted and grew into adult plants with flowers, still the F_1. The adults were then self-pollinated and produced seeds in turn. These constituted the young of the second generation, or F_2. Mendel got a total of 8,023 seeds in the F_2 in his experiments. Of these, 6,022 were yellow, 2,001 were green. This is a very close approximation to the 3 : 1 ratio.

Mendel also crossed a race with round seeds to one with wrinkled seeds. Round is probably normal and wrinkled a mutation. We can label the mutant gene w and its normal allele $+$ (again not the same $+$ as the previous ones). The P_1 were then $+/+$ (round) $\times$ w/w (wrinkled) and the F_1 were $w/+$. In appearance the F_1 were round. This makes the normal allele ($+$) dominant. When the F_1 were self-fertilized, they produced 5,474 round and 1,850 wrinkled, or almost exactly 3 round : 1 wrinkled in the F_2.

A host of research workers in many different countries following upon Mendel have found that his principle applies to virtually all forms of life, both plant and animal.

A Case of Intermediate Expression in the Hybrid.—In all Mendel's crosses, one allele was dominant to the other (the normal allele usually being dominant, the mutant recessive). The rule of dominance and recessiveness holds in many cases but not in all. It does not hold for the flower color of four-o'clocks (*Mirabilis jalapa*). There are different varieties of four-o'clocks, including one which has red flowers, another which has white. A cross of the two varieties produces hybrids that have pink flowers. When the hybrids are self-fertilized they produce offspring in the ratio of 1 red : 2 pink : 1 white.

The above cross has essentially the same explanation as Mendel's pea crosses, except that there is no dominance. The plants with the red flowers are probably normal and those which have white flowers are a mutant race. We can indicate the mutated gene by w and its normal allele by $+$. The gametes of the red race all contain the $+$ allele; those of the white race the w allele. The hybrids are $w/+$. Neither $+$ nor w is dominant but each allele has its influence on development and the hybrid is pink—intermediate in color. In the F_2 the *genotypic* ratio is $1 +/+ : 2 w/+ : 1 w/w$, which is the same sort of genotypic ratio as Mendel got in his peas. In the present instance the hybrids ($2 w/+$ or 2 pink) are distinct in appearance from either pure type ($+/+$ or red and w/w or white), so that the *phenotypic* ratio is 1 red : 2 pink : 1 white.

It might seem at first sight as though the normal and the white allele had mixed with each other in the hybrid four-o'clock and become pink. But this is not true. If they had mixed the hybrid could have produced nothing but pink offspring. The fact that it can produce pure red and pure white offspring shows that the alleles do not mix. The hybrid must be forming two types of gametes, pure $+$ and pure w. The F_2 offspring of pure type (red or white) are formed when gametes of like type come together (two $+$'s or two w's). The F_2 pinks are formed when w and $+$ come together. They are of the same genotype as the F_1 pink hybrids ($w/+$), and like them are capable of producing pure red and pure white offspring. The hybrids, in brief, appear pink but they contain the color alleles in pure form.

The color of the hybrid four-o'clocks might be compared to the pink light which would result if lights from red and white lamps were thrown on a screen at the same time. In this case there is no mixing at the source; the red and white lamps are themselves not changed. In the same way the normal and white alleles in the four-o'clocks give rise to a mixed expression but the alleles themselves do not mix; otherwise the hybrid could not produce pure red and pure white offspring.

Erroneous Ideas Concerning Mendel's Principle.—When Mendel's discoveries first came to the attention of biologists there was confusion as to their real significance. Some said that the Mendelian principle was the "law" of dominance. This was soon seen not to be true when four-o'clocks were crossed. Other forms of life were also crossed and found to show intermediate expression

in the hybrid. Then again it was said that the Mendelian principle was the 3 : 1 ratio. But this ratio is got only when two hybrids are interbred and when there is dominance. The principle really at the bottom of Mendel's results is the segregation of alleles without previous admixture in the hybrid. This results in the formation of two classes of gametes in the hybrid. The particular ratio which is then got among the offspring of the hybrids depends in part on how the hybrids are bred—whether to one another or to one of the pure parental races. It also depends on whether or not there is dominance. There was no confusion in Mendel's own mind as to the significance of his results.

The Practical Importance of Dominance.—The fact that there is dominance and recessiveness in so many cases of heredity is of importance from a practical standpoint. Take idiocy in illustration. Idiocy has various causes, but sometimes it is due to a recessive mutant gene. We can designate the mutant gene as i and its normal allele as $+$. A person who was hybrid for idiocy would appear perfectly normal. If he married a normal woman of pure type all of his children would be normal. For he would be of genotype $i/+$; his wife $+/+$. All of the children would get the $+$ allele from his wife. Half would get his i allele, the other half his $+$ allele. In other words, $i/+ \times +/+$ (parents) gives $1\ i/+ : 1\ +/+$ (offspring). Thus if the hybrid's wife were a pure normal then all the children would appear normal, although half of them would be hybrids. If the hybrids in turn married pure normals all the children again would be normal appearing, though half would be hybrids. Thus the idiot gene might exist for any number of generations in hybrids without expressing itself, and a hybrid normal might scan back over his family tree for many generations without finding an idiot ancestor. But if he married a woman of similar family history he might have some idiot children. For both parents would now be hybrids ($i/+ \times i/+$), and a quarter of the children would on the average be idiots, since $i/+ \times i/+$ gives children in the ratio of $1\ +/+ : 2\ i/+ : 1\ i/i$, or 3 normals : 1 idiot. Thus though neither parent might have suspected idiocy either in himself or his ancestors, yet they might have an idiot child.

The Fundamental Importance of Mendel's Principle.—It is really a remarkable fact that alleles do not mix, if one stops to think about it for a moment. In a person hybrid for idiocy ($i/+$) the idiot gene and its normal allele exist together in the microscopic

dimensions of every unreduced cell from the time the hybrid starts
life as a fertilized egg until the time that he forms his sperm cells.
Yet the one does not mix with the other. The normal allele is just
as good when it leaves the hybrid as when it entered him, and the
idiot allele is just as bad. The two alleles might continue to be to-
gether in hybrids generation after generation, yet without per-
manent influence on each other. One might naturally think that
the normal allele would not be as good nor the defective as bad
after many generations of hybrids, but this is not so. Mendel's
principle tells us alleles do not mix, regardless of the number of
generations they are together. This obviously is a matter of funda-
mental importance in heredity.

Simple Mendelian Inheritance Versus Complicated.—
When a geneticist asks "How is a trait inherited?" what he has in
mind usually is some trait different from the normal, and what he
wants to know among other things is whether the trait is due to
one or to several mutations. If the trait is due to one mutation,
then parents hybrid for the trait are simple hybrids (or hybrid at
just *one* locus) and they yield offspring in the *simple* Mendelian ratio
(3 : 1). If the trait represents a combination of two or more muta-
tions, then parents hybrid for the trait are *complex* hybrids (or
hybrid at more than one locus), and they yield offspring in *com-
plicated* Mendelian ratios. Just how complex the hybrid is can be
found out from the complexity of the ratio, as we shall later see.

We do not usually ask how a *normal* trait is inherited, because
we realize that any normal trait is due to the interaction of a great
many genes, and it would be next to impossible to tell how many of
these there were. What we really study in heredity is the transmis-
sion of mutant genes and their normal alleles to the offspring of
hybrid parents. That is why we center our interest on mutant
traits in the study of heredity.

Cases of Simple Mendelian Inheritance in Man.—Hun-
dreds of abnormal traits are now known in man which show simple
Mendelian inheritance, and the list of such traits is constantly
growing. Many of these abnormalities involve such things as ner-
vous disorders, peculiar skin diseases, anatomical deformities,
constitutional weaknesses which predispose the individual to ail-
ments of various kinds, and so on. All of these are due to mutations.
Often they are very rare abnormalities, no doubt because the mu-
tation has not as yet had time to spread or because it has caused a

high early mortality rate in persons in which it expresses itself, or has otherwise hindered them from reproducing.

It must not be thought, however, that only abnormalities are inherited according to Mendel's principle. The inheritance of a gene cannot be followed until it mutates and differs from its normal allele. Only then can hybrids come into existence (by the crossing of mutant and normal) and only then can it be shown that Mendelian segregation is taking place. It so happens that most mutations cause defects and therefore most cases of simple Mendelian inheritance known in man are concerned with defects. But presumably any mutated gene, even if not for a conspicuous abnormality, would segregate from its normal allele in the hybrid, and would therefore show Mendelian inheritance. Moreover, defects attract attention and therefore pedigrees are often kept of families that show them. This would be another reason why their inheritance should so often be known.

The Determination of Dominance and Recessiveness in Man from a Study of Pedigrees.—It is very desirable to find out whether a given trait is dominant or recessive, but this is often difficult in the case of man. Take the following pedigree in illustration. One parent has myopia (near-sightedness); the other is normal. Half of the children have myopia; half are normal. Which now is dominant, the abnormal trait (myopia) or the normal trait (normal sight)? So far the pedigree by itself goes, myopia might be either dominant or recessive. This is evident from the following possible genotypes of the parents and offspring.

	Parents	*Offspring*
	Myopic × normal	1 myopic : 1 normal
Myopia dominant.......	$M/+ \times +/+$	$1\ M/+ : 1\ +/+$
Myopia recessive.......	$m/m \times m/+$	$1\ m/m + 1\ m/+$

If myopia is dominant then the myopic parent is the hybrid, and the normal parent a pure recessive (upper formula shown above). If on the other hand myopia is recessive, then the normal parent is the hybrid and the myopic parent the pure recessive (lower formula). Hence if we are not given the genotypes but merely the *appearance* of the parents and the offspring (the phenotypes), then all that the pedigree under discussion tells us is that one parent is a hybrid, the other a pure recessive. It does not, however, tell us which parent is the hybrid, nor which is the pure

recessive. In general, we cannot determine dominance and recessiveness merely on the basis of the phenotypes when we are given a pedigree in which one parent is abnormal, the other normal, and in which half the children are abnormal and the other half normal.

However, we might be able to tell whether the abnormality was dominant or recessive if we had some other information in addition to the above phenotypic pedigree. Suppose, for example, that the abnormal trait were very *rare*. Suppose further the normal parent were *unrelated* to the abnormal. Then it would be very unlikely that the normal parent would be carrying the gene for the abnormality, since the gene is rare in the population at large. Hence the parents would be $A/+$ (abnormal) $\times$ $+/+$ (normal), and the abnormality would be a dominant. But if the normal appearing parent were a relative of the abnormal parent, then he (the normal) might very well be carrying the abnormal gene (since the gene is in the family). In this event the normal parent might be hybrid and the person with the abnormality a pure recessive, thus $a/+ \times a/a$. But the normal parent, though related to the abnormal, would not necessarily be carrying the abnormal gene. In this event, the abnormal person would be the hybrid and the normal-person the pure recessive, thus $A/+ \times +/+$. We could therefore have drawn no conclusion from the pedigree under discussion had the normal parent been a relative of the one with the abnormality. The same uncertainty would have obtained even if the parents were unrelated, but if the abnormality were very common in the population at large, such as is myopia. For in this case there would be a fairly good chance that a normal-appearing person carried the abnormal gene and was a hybrid.

In summary, then, assume a pedigree in which one parent is abnormal and the other normal, and in which some of the offspring are abnormal and some normal. Then the abnormality would probably be a dominant, provided the abnormality were rare and the parents unrelated. But we could not tell whether the abnormality was dominant or recessive if (1) the parents were related, even though the abnormality were rare (2) if the abnormality were common, even though the parents were unrelated.

It will be seen, then, that human pedigrees taken by themselves do not always tell us whether a trait is dominant or recessive. But sometimes they do. We might consider a few such pedigrees.

Suppose two normal persons had an abnormal child. We should then know that both parents must have been hybrid. But since they appear normal, the normal allele must be dominant, the abnormal allele recessive. Likewise, if two abnormal parents have a normal child, then again the parents must have been hybrid, but in this case the abnormal allele is the dominant.

When a trait is recessive, persons who show the trait must be pure for it, for if they were hybrids they would contain the dominant allele as well as the recessive, and they would not show the recessive trait. Hence if two parents have a recessive abnormality, both must be pure for it and all their children will have the abnormality. However, if both parents have an abnormality and all the children also have it, it does not necessarily follow that the trait is a recessive, for two reasons. One is that the trait might have been dominant and the parents, even though hybrids, might not have produced any offspring of the recessive type just as a matter of chance. This might very well happen in a small family. Again, the trait might have been dominant and both parents might have been pure for it, or one might have been pure and the other hybrid. In either event all the offspring would have the trait.

But suppose now that we had examined a great *many* pedigrees in which both parents had an abnormal trait, and suppose further that in every instance it was found that all the children had the abnormality. Then we *could* conclude that the trait was a recessive. For assume (contrary to fact) that it was a dominant. Then in at least *some* of the cases we should expect just as a matter of chance that both parents would have been hybrids, and we should therefore expect that the offspring would sometimes have been normal as well as abnormal. Hence if the children were *always* abnormal when both parents were abnormal, the trait would in all probability be recessive.

When an abnormality is a recessive it may show up in a family tree after having skipped appearing for a number of generations, as in the case of idiocy. Such skipping is not possible in the case of a dominant abnormality. No generation that carries the abnormal allele would fail to show the abnormality. It might of course happen that a parent with a dominant abnormality was a hybrid and that none of his children happened to get his abnormal gene. If they also did not get it from the other parent, the abnormality would not show up among the offspring. Neither would it show up

in some later generation unless a visibly abnormal married into the stock.

Animal and Plant Genetics in Relation to Man.—When conducting breeding experiments with animals it is desirable to choose animals of a kind that are very prolific and that reach maturity in a short time—such animals as mice, rabbits, guinea pigs, or, still better, insects. These are desirable because in breeding work we often have to get large numbers of offspring from a single mating in order to get numbers that are significant, and we must often run our experiments over several or many generations in order to see what happens from one generation to the next.

Plants afford very convenient material for the study of heredity because many plants reproduce in a year or less, and a single plant produces many seeds. Then, too, a single plant as a rule is both male and female, and we can see what kind of offspring a plant produces all by itself by simply self-fertilizing the plant, as Mendel did when he determined the genotypes of the F_2 produced by his crosses.

It would indeed have been difficult for Mendel to have arrived at his principle if he had confined himself to man. Nevertheless man is an extremely important creature. But once the general principles have been discovered in the lower forms of life, we are better prepared to study ourselves.

SUMMARY

1. Mendel's principle is the non-mixing of alleles in the hybrid. Therefore the hybrid forms two pure classes of gametes in equal proportions, such as pure yellow and pure green in peas. These by their combination produce F_2 in the 3 : 1 ratio.

2. Alleles are contained in the chromosomes and therefore they are distributed in the same way as the chromosomes at the reduction division.

3. A mutant gene occupies the same locus as the normal gene from which it arose, and therefore the two genes are alleles.

4. Often one allele does not express itself in the hybrid. It is said to be recessive; the other allele, dominant. Usually the normal allele is dominant.

5. Sometimes there is intermediate expression of alleles in the hybrid, as when a red four-o'clock is crossed to a white and produces a pink hybrid. In these cases the hybrid can produce offspring of pure type, thus showing that the alleles themselves do not mix.

6. The F_2 offspring from a cross can be classified either according to their appearance (as 3 yellow : 1 green) or according to their genetic constitution (as $1 +/+ : 2 \ g/+ : 1 \ g/g$). Classes based on appearance are referred to as *phenotypes;* those based on genetic constitution, as *genotypes.* Correspondingly, F_2 ratios are referred to as phenotypic or genotypic ratios.

7. When two hybrids are crossed, their gametes combine according to chance expectation. The 3 : 1 ratio represents an average result of these combinations, based on large numbers of offspring.

8. Mendel's principle has been demonstrated in numerous forms of life, both plant and animal.

9. Mendel's discovery that alleles do not mix was revolutionary. It disproved the notion, prevalent in Mendel's day, that there is always a complete mixture of germ plasms in a hybrid.

10. The dominance and recessiveness of alleles, though not a universal principle, is a matter of great practical importance, since it is important to know, for example, that a person might be carrying the allele for idiocy and yet appear perfectly normal.

11. By simple Mendelian inheritance is meant the transmission of a single pair of differing alleles to the offspring of a hybrid, the remaining pairs not differing. More complicated inheritance involves the transmission of more than one pair of differing alleles.

12. In man many cases of simple Mendelian inheritance are concerned with abnormalities. This is due partly to the fact that a single mutation often causes a conspicuous abnormality. Inheritance in such a case then concerns only the mutant gene and its normal allele.

13. It is often difficult to tell, from a human pedigree, whether an abnormality is dominant or recessive. Thus, suppose we were given the following pedigree. One parent is myopic, the other normal. Half the children are myopic, half normal. Then myopia might be either dominant or recessive. If dominant, the myopic parent is hybrid and the normal parent pure normal ($M/+ \times +/+$). If myopia is recessive, the myopic parent is pure and the normal parent hybrid ($m/m \times +/m$).

PROBLEMS

1. In Drosophila gray body ($+$) is dominant to black (b). A gray fly is crossed to a black ($+/+ \times b/b$). The F_1 are inbred ($+/b \times +/b$), and they produce 400 offspring in the F_2. Assume that all of the F_2 classes of offspring are equally viable (equally strong and capable of development to the adult stage). Tell how many of the F_2 on the *average* would be expected to be (1) $+/+$, (2) $b/+$, (3) b/b. Tell how many would appear gray and how many black (on the average).

2. Suppose we crossed a pure gray fly by a black, inbred the F_1, and counted 400 offspring in the F_2. Would necessarily exactly 300 of these be gray and 100 black? Give the reason for your answer.

3. Two gray flies are bred with each other and they produce 158 grays and 49 blacks. Give the genotypes of the parents (the two gray flies) and the reason for your answer.

4. Suppose we reared 400 F_1 offspring from the first cross $(+/+ \times b/b)$. What would the genotype of each one be? Would the F_1 be any more variable genetically than either parent race? Why or why not? Would the F_2 be more variable than either parent race or than the F_1? Why or why not?

5. A hybrid gray fly is crossed to a black $(b/+ \times b/b)$. Give (1) the gametes of these two parents, (2) the genotypic ratio in which their offspring are produced, (3) the phenotypic ratio.

6. A pure gray fly is crossed to a gray fly that is hybrid for black $(+/+ \times b/+)$. Give the genotypic ratio in which the offspring are produced and give their appearance.

7. If two gray flies had just one offspring and this were black, what would the genotypes of the two gray parents necessarily be? Why?

8. In *Mirabilis* (the four-o'clock), a plant hybrid for red flowers $(+)$ and white (w) is pink (i.e., $w/+$ is pink). One pink plant is crossed to a red, another to a white. Give the genotypic and phenotypic ratios in which the offspring are produced in each case.

9. There is a race of chickens known as "Andalusians," some of which have black feathers, others white. Consider black as the normal $(+)$ and white as a mutant (w). When black is crossed by white $(+/+ \times w/w)$, the F_1 hybrid $(w/+)$ is a slaty blue (neither black nor white being dominant). Give the F_2 genotypic and phenotypic ratios when the F_1 are interbred $(w/+ \times w/+)$. What cross in plants does the present case resemble in principle, as regards expression of alleles in the hybrid?

10. Would it be possible to get true breeding races of either pink four-o'clocks or blue Andalusian chickens (without further mutations)? Why or why not?

11. In man brown eyes $(+)$ are dominant to blue (b). Two brown-eyed people have a blue-eyed child. What are the genotypes of the parents?

12. Both parents of a blue-eyed man are brown-eyed. He marries a brown-eyed woman, one of whose parents was brown-eyed, the other blue, and who had a blue-eyed brother. The man and woman in question have a brown-eyed child. Give the genotypes of (1) the parents of the man and woman, (2) the man and woman, (3) their child.

13. People differ in their ability to taste, and sometimes this difference has an hereditary basis. Thus most people (about 70 per cent) get a bitter taste from the drug phenyl-thio-carbamide. About 30 per cent of people

get no taste from the drug. The "non-tasters" are due (probably) to a recessive mutation. Designate the mutant gene as n and its normal allele as $+$. A "taster" marries a "non-taster." Some of their children are tasters, others non-tasters. Give the genotypes and phenotypes of the parents and of the children.

14. In chickens "rose" comb (R) is dominant to "single" ($+$). Suppose we crossed a rose comb chicken to a single, and some of the offspring were single. What would be the genotype of the rose-comb parent, R/R or $R/+$? Suppose we did not know whether or not a rose-comb chicken carried single ($+$). How might we determine this?

15. The breed of chickens known as Wyandottes are usually pure for rose comb, and breeders prefer that they be pure. But sometimes rose comb Wyandottes carry single. How might we determine that a pair of Wyandottes to be used for breeding did not contain single comb?

16. In sheep black is recessive to white. A farmer has a large flock of sheep (several hundred), all of which are white. Suppose a small per cent (say, 2 or 3 per cent) carried black ($b/+$). Would the flock as a rule produce any black sheep? Tell why or why not. Might it occasionally produce a black sheep? Tell why.

17. In sheep a mutation caused the loss of horns in both males and females. The mutant gene is dominant to its normal allele in females but recessive in males. Designate the mutant gene as P (polled or hornless) in females, p in males, and the normal allele as $+$ (in either female or male). Assume that a pure normal male is crossed to a pure hornless female. Give the P_1 (genotypes and phenotypes, including sex), the F_1 (classify males and females separately and give both the genotypes and phenotypes), and the F_2 (produced by breeding together the F_1 males and females). In the F_2, give the F_2 genotypic and phenotypic ratios first for the males, then for the females.

18. In man baldness is sometimes due to a mutation, and the mutant gene is dominant in males, recessive in females (bald women usually conceal their baldness). Designate the mutant gene as B in males, b in females, and the normal allele as $+$. A man and his wife are both hybrid for baldness. Give their genotypes and phenotypes, and the genotypic and phenotypic ratio in which their children would be produced. First give the ratios for the male children, then for the female. Tell why baldness is less common among women than men.

4. *INDEPENDENT ASSORTMENT*

Up TO the present we have dealt with crosses which involved only one pair of chromosomes and one pair of genes. But a cross might involve two pairs of chromosomes (Fig. 22). The resulting offspring is hybrid at two loci and can be referred to as a *di-hybrid*.
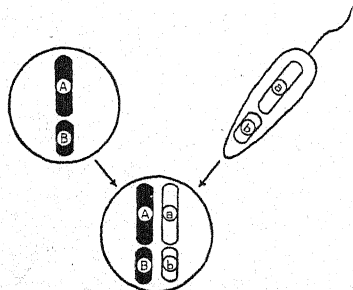
Before the di-hybrid forms its gametes, the chromosomes come together in pairs and the reduction division takes place as usual. But note the two possible ways in which the pairs might be arranged at metaphase (Fig. 23). The two chromosomes of maternal origin (black in Fig. 23) might be on one side of the equatorial plate and the two paternal (light) on the other; *or*, the two in each case might be on opposite sides. As a result the di-hybrid forms four classes of gametes (*AB*, *ab*, *Ab*, *aB*). These four are formed in equal proportions because the two possible arrangements at metaphase occur with equal frequency on the average.

Fig. 22. The di-hybrid.

Inspection of Fig. 23 will show that one member of each chromosome pair goes to every reproductive cell of a hybrid, but it is a matter of chance whether members of two pairs go to the same cell or to opposite cells at the reduction division. In short, one pair of chromosomes is assorted *independently* of any other pair. This applies because one pair is not tied to the other but the two are entirely independent of each other. We might then define independent assortment as the segregation of one pair of chromosomes (or one pair of genes) without reference to a second pair.
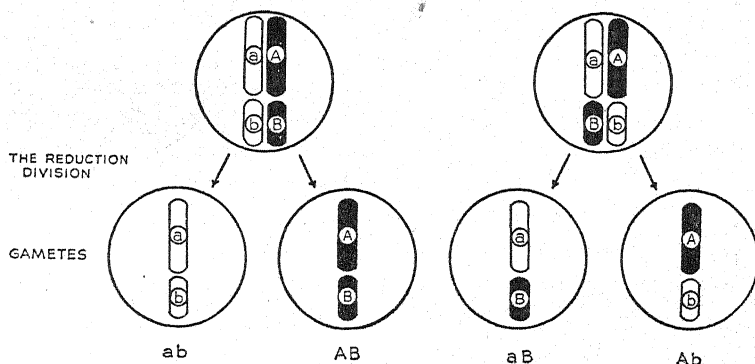
Fig. 23. Independent assortment.

The 9 : 3 : 3 : 1 Ratio.—Mendel discovered independent assortment. This he did by means of crosses between different races of peas. For example, Mendel crossed a race of peas with yellow
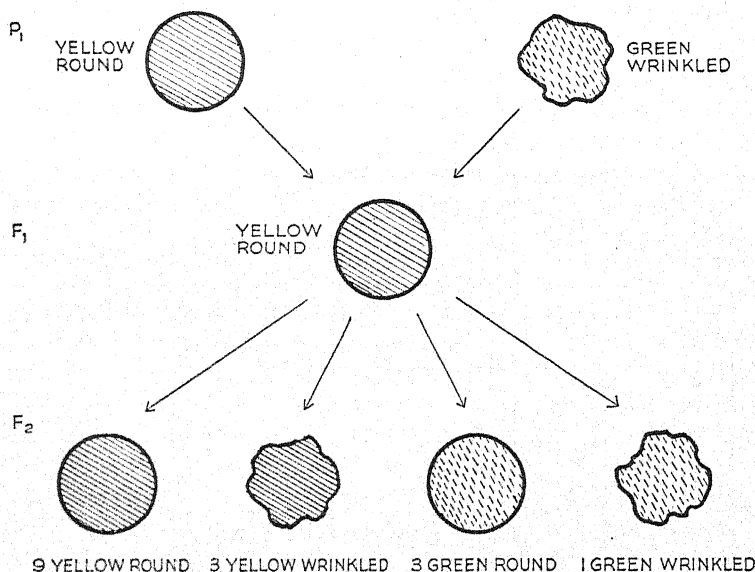
Fig. 24. The cross of a yellow round by a green wrinkled pea.

round seeds by a green wrinkled variety (Fig. 24). The F_1 were yellow round (yellow and round being dominant to green and wrinkled). The F_1 upon being self-fertilized produced F_2 in the

ratio of 9 yellow round : 3 yellow wrinkled : 3 green round : 1 green wrinkled.

We can explain the above cross in terms of chromosomes and genes. Yellow round is the normal race and green wrinkled contains two recessive mutations, each in a different kind of chromosome. We can indicate the mutant genes by g (green) and w (wrinkled), and their normal alleles by $+$ signs, the $+$'s at different loci representing *different* genes (Fig. 25, top). The genotype of the pure normal is $\dfrac{+\ +}{+\ +}$ and of the green wrinkled $\dfrac{g\ w}{g\ w}$. The F_1 produced by crossing the two are of genotype $\dfrac{g\ w}{+\ +}$. (In these formulae the two members of a given gene pair are placed above and below a given line.) The normal alleles are dominant and so the F_1 $\left(\dfrac{g\ w}{+\ +}\right)$ appear yellow round. Before the F_1 form their gametes, the reduction division takes place (Fig. 25). As a result the F_1 produce four classes of gametes; namely, $+\ +$, $g\ w$, $+\ w$, $g\ +$, or to use a different arrangement, $+\ +$, $+\ w$, $g\ +$, $g\ w$.

When the F_1 are self-fertilized their sperm and egg cells combine in various possible ways. For example, sperm cells of class $+\ +$ might combine with any one of the four classes of eggs ($+\ +$, $+\ w$, $g\ +$, $g\ w$), giving four classes of fertilized eggs: $\dfrac{+\ +}{+\ +}, \dfrac{+\ w}{+\ +}, \dfrac{g\ +}{+\ +}, \dfrac{g\ w}{+\ +}$ (where the genes derived from the eggs are placed above the lines, those from the sperm cells below). But there are four classes of sperm cells in all ($+\ +$, $+\ w$, $g\ +$, and $g\ w$), and it is possible for any one of these classes to fertilize all four classes of eggs. We might make these combinations by first placing the four classes of eggs along the upper side of a "checkerboard" and the four classes of sperm cells along the left side, as shown in Fig. 25. The first class of sperm cells are then combined with the four classes of eggs and the resulting combinations (fertilized eggs) placed in the uppermost horizontal row of squares. The combinations of the second class of sperm cells with the four classes of eggs are placed in the second horizontal row, and so on. In all there are sixteen possible combinations, since each of the four classes of sperm cells combines with each of the four classes of eggs. The method just given of making the combinations
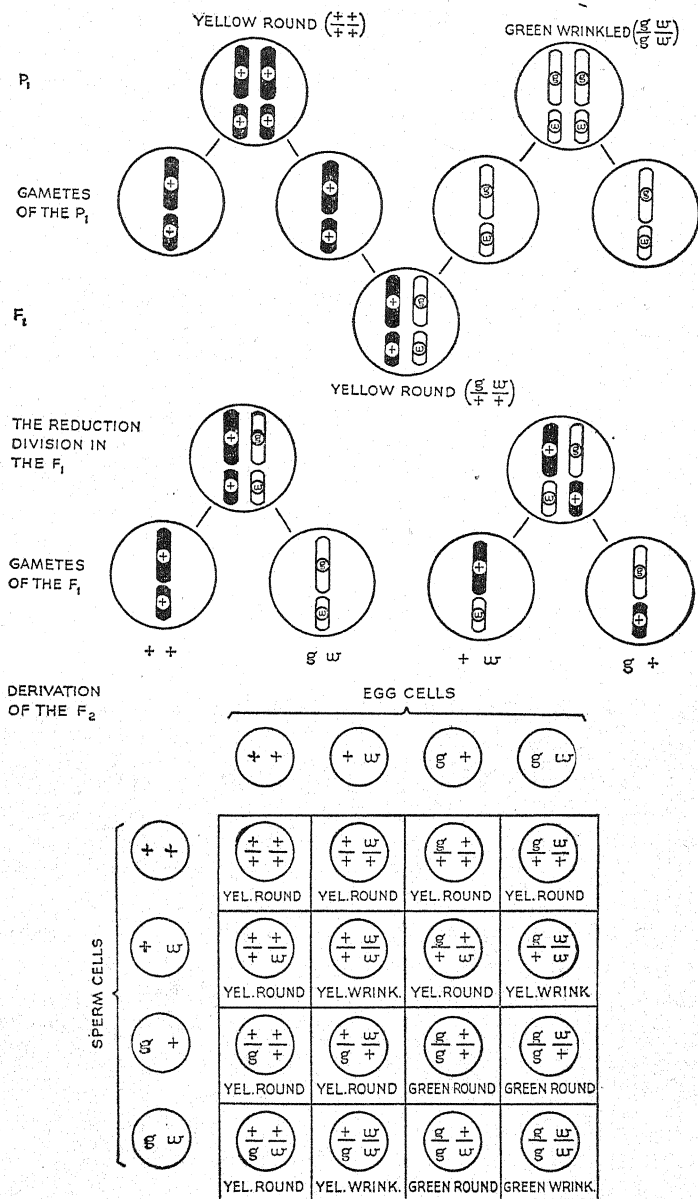
FIG. 25. The explanation of the cross of a yellow round pea by a green wrinkled pea.

between the sperm cells and eggs is known as the "checkerboard" method. We might, however, simply have put the eggs on one horizontal line, the sperm cells on another line directly below, and then have "multiplied" sperm cells by eggs by making the combinations in essentially the manner just described.

Not all of the combinations shown in Fig. 25 contain different kinds of genes. Thus $\frac{g}{+}\frac{+}{w}$ (second horizontal row, third square) has the same kinds of genes as $\frac{+}{g}\frac{+}{w}$ (fourth row, first square). Accordingly, both $\frac{g}{+}\frac{+}{w}$ and $\frac{+}{g}\frac{+}{w}$ develop into the same type of offspring (yellow round). There are also different genotypes that will look alike. Thus $\frac{+}{+}\frac{+}{+}$ will be yellow round (normal), but so will $\frac{+}{g}\frac{+}{w}$, because both + and + are dominant, respectively, to g and w. If one adds up the seeds that are yellow round, he will find nine in all. Again, seeds of genotype $\frac{+}{+}\frac{w}{w}$ will appear yellow wrinkled, and so will those that are $\frac{g}{+}\frac{w}{w}$; if we added up all seeds that appear yellow wrinkled, we should find three in all. In like manner we should find that there were three green round and that there was one green wrinkled. That is, by lumping together all seeds of like *appearance* we should get a phenotypic ratio of 9 yellow round : 3 yellow wrinkled : 3 green round : 1 green wrinkled. This is the ratio which Mendel approximately got in the F_2 when he inbred hybrids produced by crossing yellow round with green wrinkled. His actual figures were: 315 yellow round, 101 yellow wrinkled, 108 green round, and 32 green wrinkled. If these figures are reduced to a ratio we get 9.06 yellow round : 2.9 yellow wrinkled : 3.1 green round : 0.9 green wrinkled. This is a very close approximation to the 9 : 3 : 3 : 1 ratio expected in the F_2 if the two pairs of alleles undergo independent assortment in the hybrid.

Methods of Deriving the 9 : 3 : 3 : 1 Ratio.—Upon adding up all the yellows and all the greens in the above 9 : 3 : 3 : 1 ratio we find that there are 12 yellow : 4 green, which is the same as 3 yellow : 1 green. Adding up all the round and wrinkled and dividing through, we get 3 round : 1 wrinkled, again the simple Mendelian

ratio. The offspring of the di-hybrid fall into the 3 : 1 ratio when either trait (seed color or seed texture) is considered by itself. But when we classify the offspring for both traits at the same time we get the 9 : 3 : 3 : 1 ratio.

From the fact that each locus by itself gives a 3 : 1 ratio in the F_2 we could directly have derived the 9 : 3 : 3 : 1 ratio for both loci combined. For when we say that there are 3 yellow : 1 green in the F_2 this is the same as saying ¾ are yellow, ¼ are green. Hence out of every 16 F_2 offspring ¾ or 12 would on the average be yellow, ¼ or 4 would be green (Fig. 26). But the 12 yellows would
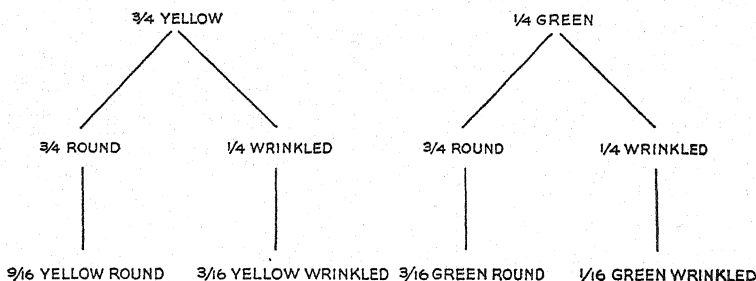
FIG. 26. The derivation of the 9 : 3 : 3 : 1 ratio by the "direct" method.

be subdivided into ¾ round and ¼ wrinkled, giving us 9 yellow round : 3 yellow wrinkled. Likewise, the four greens would be subdivided into ¾ round and ¼ wrinkled, giving us 3 green round : 1 green wrinkled. All this of course is based on the hypothesis that the two pairs of alleles undergo independent assortment.

It will be seen, then, that we can derive the 9 : 3 : 3 : 1 ratio in either of two ways. We can derive it indirectly by "multiplying" the four classes of sperm cells with the four classes of eggs formed by the F_1 hybrid. This might be referred to as the *indirect* method of deriving the F_2. On the other hand we can derive the F_2 ratio directly by combining the 3 : 1 ratios for each locus—the *direct* method. However, the direct method is merely a short-cut. The gametes still combine to produce the F_2, but for each locus separately they produce the 3 : 1 ratio, and by simply combining the two 3 : 1 ratios we get the 9 : 3 : 3 : 1 ratio.

The Test Cross.—Suppose now that we crossed the di-hybrid to a green wrinkled, thus $\dfrac{g}{+}\dfrac{w}{+} \times \dfrac{g}{g}\dfrac{w}{w}$. What kind of offspring

would this cross produce? The hybrid would as before form four classes of gametes $(+ +, + w, g +, g w)$. But the green wrinkled parent $\left(\dfrac{g\ w}{g\ w}\right)$ would form only one class of gametes $(g\ w)$. To derive the offspring we should therefore simply add $g\ w$ to each of the four classes of gametes produced by the hybrid, giving us four classes of offspring in the ratio of $1\ \dfrac{+\ +}{g\ w} : 1\ \dfrac{+\ w}{g\ w} : 1\ \dfrac{g\ +}{g\ w} : 1\ \dfrac{g\ w}{g\ w}$ (where the genes derived from the hybrid are shown above the horizontal lines and those derived from the green wrinkled parent below).

In the above cross the appearance of each class of offspring tells us directly what genes came from the hybrid parent. For example, the third class of offspring listed above $\left(\dfrac{g\ +}{g\ w}\right)$ appears green round. Since it is green it must have got g from both parents, and hence one g from the hybrid. Since it is round it must have the $+$ allele. This could not have come from the green wrinkled parent, since this parent contributes only $g\ w$ to the offspring. It must therefore have come from the hybrid parent. Thus the appearance of the offspring in question corresponds to the genes which it receives from the hybrid parent. The same sort of thing is true of the remaining offspring. In the cross under consideration, then, the offspring correspond in appearance and proportions to the classes of gametes produced by the hybrid parent. This cross can therefore be regarded as a *test* of the di-hybrid's gametes and is accordingly referred to as a *test cross*. It is also sometimes referred to as a *back cross* from the fact that the hybrid is being crossed back to one of the parent races—in the present example to the green wrinkled race. In general terms we can define a test cross as a cross between a hybrid parent and a recessive parent.

Mendel made test crosses of the di-hybrid $\left(\dfrac{g\ w}{+\ +} \times \dfrac{g\ w}{g\ w}\right)$ and got the following numbers of offspring: 55 yellow round $\left(\dfrac{g\ w}{+\ +}\right)$, 49 yellow wrinkled $\left(\dfrac{+\ w}{g\ w}\right)$, 51 green round $\left(\dfrac{g\ +}{g\ w}\right)$, 53 green wrinkled $\left(\dfrac{g\ w}{g\ w}\right)$. These numbers when reduced to a ratio are a close approxi-

mation to $1 \dfrac{+\ +}{g\ w} : 1 \dfrac{+\ w}{g\ w} : 1 \dfrac{g\ +}{g\ w} : 1 \dfrac{g\ w}{g\ w}$. This ratio indicated that the di-hybrid formed four classes of gametes in equal numbers and this in turn meant that the two pairs of alleles underwent independent assortment in the hybrid. The 9 : 3 : 3 : 1 ratio indicates the same thing, but not quite as directly as the test cross.

It is to be emphasized that Mendel did not know in advance of his experiments that the two pairs of alleles underwent independent assortment in his hybrids but that he first made his crosses, got hybrids, and found that the hybrids produced offspring approximately in the 9 : 3 : 3 : 1 ratio when inbred; or, as a still better proof, in the 1 : 1 : 1 : 1 ratio when test crossed.

Mendel's Experiments and Linkage.—Mendel made several crosses involving two pairs of alleles. It so happened, in all his experiments, that he dealt with genes in different pairs of chromosomes, and in all cases he got the 9 : 3 : 3 : 1 ratio in the F_2 upon inbreeding the hybrids, or in the 1 : 1 : 1 : 1 ratio upon making the test cross. But one chromosome contains more than one gene, and had Mendel been dealing with genes at different loci in the same chromosome, he would have got a different result from the one he obtained. For a chromosome tends to be handed down in its entirety to the offspring, and all genes that are in the same chromosome tend to be handed down to the offspring in a group. They are *tied* to one another and are said to be *linked*. It is only when pairs of alleles are in separate pairs of chromosomes that they are assorted independently of each other. But Mendel discovered a very important thing about genes when he found that alleles do not mix with each other in the hybrid. This, let it be repeated, is fundamentally the Mendelian principle.

Mendel's First and Second Principles.—The non-mixing of alleles in the hybrid is sometimes referred to as *Mendel's first principle*, and independent assortment as *Mendel's second principle*. Up to the present there is no experimental evidence indicating that alleles ever mix in the hybrid, and so Mendel's first principle is truly a principle in that it seems to be of universal application. But two or more pairs of genes are not always assorted independently of one another, since genes are often linked. Therefore we cannot really refer to independent assortment as a principle, if we are to use this term to mean a universal law. Nevertheless independent assortment is a very important fact in heredity.

The Tri-hybrid.—In peas dwarf (d) is recessive to tall ($+$) and is in a separate chromosome from either green or wrinkled. A plant might be hybrid not only for green and wrinkled $\left(\dfrac{g\ \ w}{+\ \ +}\right)$ but also for dwarf, thus $\dfrac{g\ \ w\ \ d}{+\ \ +\ \ +}$. This plant is hybrid at three loci and can

A. (DI-HYBRID)

(SHOWN TWICE BELOW)   (SHOWN TWICE BELOW)

B. (TRI-HYBRID)

| $++ +$ | $g\,w\,d$ | $++d$ | $g\,w+$ | $+w+$ | $g+d$ | $+w\,d$ | $g.++$ |

Fig. 27. The reduction division in the di-hybrid and the tri-hybrid compared. In the upper part of the diagram (A) only two pairs of chromosomes are considered. In the lower part (B) the third pair is added (below the broken line).

be referred to as a tri-hybrid. It might be produced by crossing a pure yellow round tall $\left(\dfrac{+\ +\ +}{+\ +\ +}\right)$ by a green wrinkled dwarf $\left(\dfrac{g\ w\ d}{g\ w\ d}\right)$.

In the di-hybrid $\left(\dfrac{g\ \ w}{+\ \ +}\right)$ we were concerned with just two pairs of chromosomes and at the reduction division these were arranged in two possible ways with regard to each other, as shown in the upper part of Fig. 27. If now the plant is hybrid in addition at a

locus in a third pair of chromosomes (at the *d* locus), then this third pair might be arranged in either of two possible ways with regard to the first two pairs (middle part of Fig. 27). In the first arrangement + is to the left and *d* to the right; in the second, the two alleles are reversed with regard to the first arrangement. This gives us four possible arrangements of the chromosome pairs and four possible types of reduction divisions. Since each division results in two classes of gametes, we get a total of eight different gametic classes in equal proportions, as shown in Fig. 27.
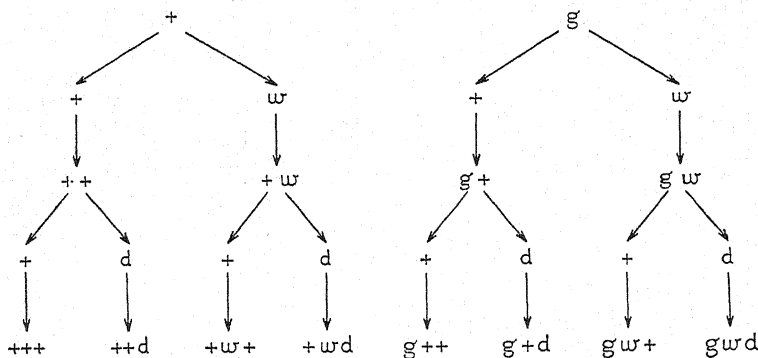
Fig. 28. The derivation of the gametes of the tri-hybrid by the "branching" method.

We could have arrived at the eight classes of gametes in a somewhat different way, as shown in Fig. 28. First we consider the plant hybrid for just the first pair of alleles (+/g). It then forms just two classes of gametes in equal proportions: + and *g*. If in addition the plant is hybrid for the second pair (+/w), then the gametes that get the first + might get either the second + or *w*; so might those that get *g*. This gives us the four classes of gametes formed by the di-hybrid, or + +, + *w*, *g* +, *g w*. If the plant is also hybrid for the third pair of alleles (+/d), then each of the four classes of gametes just given might get either the third + or *d*, giving the eight classes shown in Fig. 28. These eight classes are formed in equal proportions.

The Offspring of Tri-hybrids.—Suppose now that we self-fertilized the tri-hybrid $\frac{g \; w \; d}{+ \; + \; +}$. Then the eight classes of sperm cells would combine with eight classes of egg cells. We might make

these combinations by first numbering the eight classes 1 to 8 (in the case of both sperm cells and eggs). We could then take sperm cells of class 1 and combine them with all 8 classes of egg cells, giving 8 combinations. Next we could do the same with sperm cells of class 2, again giving us 8 combinations, or 16 so far. Then we could continue until we would have taken all 8 classes of sperm cells with the 8 classes of egg cells. This would give us 8 × 8 or 64 possible combinations between the sperm cells and egg cells.

FIG. 29. The derivation of the tri-hybrid ratio (direct method).

We could derive the offspring of the tri-hybrid without going to the trouble of multiplying out the sperm and egg cells. In the cross yellow round × green wrinkled we saw that $\frac{3}{4}$ of the F_2 were yellow and $\frac{1}{4}$ green and that each of these classes could be subdivided into $\frac{3}{4}$ round and $\frac{1}{4}$ wrinkled, giving $\frac{9}{16}$ yellow round, $\frac{3}{16}$ yellow wrinkled, $\frac{3}{16}$ green round, $\frac{1}{16}$ green wrinkled (Fig. 29). If the plant is hybrid in addition at the third locus ($+/d$), then each of the above 4 classes can in turn be divided into $\frac{3}{4}$ tall and $\frac{1}{4}$ dwarf. Dividing the $\frac{9}{16}$ yellow round into $\frac{3}{4}$ tall and $\frac{1}{4}$ dwarf, we get $\frac{27}{64}$ yellow round tall and $\frac{9}{64}$ yellow round dwarf (Fig. 29). We could do likewise for the remaining three classes. All told, we should get eight classes of offspring, as shown in Fig. 29.

The Test Cross of the Tri-hybrid.—A green wrinkled dwarf pea plant $\left(\dfrac{g\ w\ d}{g\ w\ d}\right)$ is pure for three recessive alleles and might therefore be referred to as a *triple recessive*. A plant that is hybrid for green wrinkled and dwarf might be crossed to the triple recessive,

thus: $\dfrac{g}{+}\ \dfrac{w}{+}\ \dfrac{d}{+} \times \dfrac{g\ w\ d}{g\ w\ d}$. This constitutes the test cross. The hybrid
parent forms the eight classes of gametes we have already derived,
but the triple recessive forms only one class of gametes: namely,
$g\ w\ d$. Therefore we can derive the offspring of the test cross by
simply adding $g\ w\ d$ to the eight classes of gametes formed by the
hybrid parent, previously shown in Fig. 28. For example, the first
class in Fig. 28 $(+ + +)$, if combined with a $g\ w\ d$ gamete would
give offspring of genotype $\dfrac{+\ +\ +}{g\ w\ d}$; the second class $(+ + d)$ with
$g\ w\ d$ would give $\dfrac{+\ +\ d}{g\ w\ d}$; and so on. The first class $\left(\dfrac{+\ +\ +}{g\ w\ d}\right)$
would appear $+ + +$ (yellow round tall); the second class
$\left(\dfrac{+\ +\ d}{g\ w\ d}\right)$ would appear $+ + d$ (yellow round dwarf); and in gen-
eral the offspring would correspond in appearance with the genes
contributed by the hybrid parent. Therefore the offspring of the
test cross tell us directly what kinds of gametes are formed by the
tri-hybrid and in what ratio they are formed. The offspring pro-
duced by self-fertilization tell us the same thing, but not directly.

In deriving the offspring of the tri-hybrid $\left(\dfrac{g\ w\ d}{+\ +\ +}\right)$, we started
out by assuming that in peas green, wrinkled, and dwarf were in
three separate pairs of chromosomes. We then derived the gametes,
and from the gametes we derived the offspring of the hybrid. But
in actual practice we proceed in the opposite direction. We first get
the offspring of the hybrid, preferably from a back cross. The off-
spring then tell us that the hybrid forms eight classes of gametes in
equal proportions, and this in turn tells us that the three pairs of
genes at the green, wrinkled, and dwarf loci are assorted inde-
pendently of one another. We then conclude that the three gene
pairs are in separate chromosome pairs.

We could have arrived at the above conclusion without getting
plants hybrid for all three pairs of alleles at the same time but by
getting them hybrid for just two of the three pairs at a time. This
can be seen from the following consideration. Let us number the
pairs of alleles 1, 2, and 3 (a given number referring to *both* mem-
bers of a given pair). There are three possible ways of taking the
three pairs of alleles two at a time; namely, 1 and 2, 1 and 3, 2 and

3. If plants hybrid for gene pairs 1 and 2 yield a 9 : 3 : 3 : 1 ratio on being self-fertilized, or a 1 : 1 : 1 : 1 ratio on being test crossed, then we know that pairs 1 and 2 are assorted independently and are in separate pairs of chromosomes. Likewise we could determine that gene pairs 1 and 3 were in separate pairs of chromosomes, and that 2 and 3 were. Thus we should have proved that all three pairs of alleles were in separate pairs of chromosomes. We could then predict in what ratio offspring would be produced by plants hybrid for all three pairs at the same time.

General Mendelian Formulae.—As a hybrid becomes more complex through the addition of further hybrid loci, its gametes become more diversified. We can go about matters in a systematic manner and derive the number of gametic classes a hybrid forms with increasing complexity. We can use the symbols A, B, C, etc., for dominant alleles and a, b, c, etc., for recessive. If a parent is hybrid at the a locus, it forms two classes of gametes in equal proportions, A and a. If it is hybrid in addition at a second locus (the b locus), it forms 4 classes, because the gametes which get A might get either B or b, and those that get a might also get either B or b. The addition of the second locus doubled the previous number of gametic classes (2). This can be expressed numerically as 2×2 or 2^2. If the parent were hybrid at a third locus (the c locus), then it would form double the previous number of gametic classes, because each of the four previous classes might receive C or c, giving us 8 gametic classes. This could be expressed numerically as $2 \times 2 \times 2$ or 2^3. The power to which the root 2 is raised is equal to the number of loci at which the parent is hybrid. In general, if the parent is hybrid at "n" loci (where n equals any number), it forms 2^n classes of gametes.

The number of combinations possible between the gametes of two hybrid parents also increases with the complexity of the hybrids in a regular manner. When the parents are hybrid at just one locus the two classes of gametes which each forms can undergo four possible combinations (resulting in the 3 : 1 ratio), as seen in any simple Mendelian cross. This number (4) is equal to the square of the number of classes of gametes (2×2, or 2^2). When the parents are hybrid at two loci each forms four classes of gametes and these can undergo sixteen possible combinations (resulting in the 9 : 3 : 3 : 1 ratio). This number 16 is equal to 4×4 (or 4^2), again the square of the number of gametic classes. In general, then, the

number of combinations possible between the gametes of the two hybrids is equal to the square of the number of classes of gametes that they form, as is readily seen in connection with the "checkerboard" method. If we let 2^n stand for the number of gametic classes, then the number of combinations possible between them is $(2^n)^2$ or 2^{2n}. Some of the resulting offspring look alike, so that the number of differently appearing offspring (or different phenotypic classes) is not equal to the number of combinations between the gametes.

What formula expresses the number of phenotypic classes in the F_2? Assuming that there is dominance, then when the parents are hybrid at just one locus they form two phenotypic classes of offspring, as yellow and green (in the 3 : 1 ratio). If they are hybrid at a second locus, say, the wrinkled locus, then each of the first two classes (yellow and green) can be subdivided into two classes (round and wrinkled), giving us four classes (in the 9 : 3 : 3 : 1 ratio). With the addition of each hybrid locus we double the previous number of phenotypic classes, since we subdivide each previous class into two on the basis of two new traits. In general, the number of F_2 phenotypic classes is equal to the root 2 raised to a power equal to the number of hybrid loci, or 2^n. This is the same as the number of gametic classes that the hybrid forms. Of course the phenotypic ratio in which the F_2 offspring are thrown is another matter. This can be got by multiplying the ratios for each separate locus, as we have seen.

Consider finally the number of genotypic classes formed by any hybrid. When we were considering the simple cross of yellow peas by green ($+/+ \times g/g$), we saw that the F_1 ($+/g$) upon being inbred produced offspring in the genotypic ratio of $1 +/+ : 2$ $g/+ : 1$ g/g. In other words, it produced three genotypic classes. If the F_1 were hybrid at a second locus, then each of these three genotypic classes would be subdivided into three on the basis of the second locus, making nine genotypic classes in all. If the F_1 were hybrid at a third locus, then each of the nine previous classes would be subdivided into three again, making twenty-seven genotypic classes. In other words, for one hybrid locus the number of genotypic classes is 3 (or 3^1); for two hybrid loci it is 3×3 or 3^2; for three it is $3 \times 3 \times 3$ or 3^3. From this it can be seen that the number of genotypic classes of offspring formed by parents hybrid at n loci is 3^n.

In summary, parents hybrid at n loci form 2^n classes of gametes, 2^n phenotypic classes of offspring, and 3^n genotypic classes of offspring. The number of possible combinations that the 2^n gametes undergo is the square of 2^n, or 2^{2n}, and these combinations result in the 2^n phenotypic, or the 3^n genotypic classes of offspring.

The High Degree of Hybridity of Man.—On the basis of the above discussion we can understand why no two children in the same family are ever alike by heredity (unless they happen to be identical twins). Man is a very hybrid creature. He has 24 pairs of chromosomes, and he is probably hybrid at one locus or another in every one of these 24 pairs. A man thus hybrid would form 2^{24} classes of reproductive cells; so would his wife. This figure (2^{24}) is equal to about 16 million. The number of possible combinations at fertilization would be the square of this (2^{48}), or roughly 250,000,000,000,000. A considerable fraction of these combinations would be different (genotypically). Thus the chances of any two combinations being alike at fertilization are extremely remote. The above large figure should be even larger, because a person might be hybrid at two or more loci in a given chromosome pair and recombinations are possible between the gene pairs in a chromosome pair (as will be considered later).

Ways of Indicating the Complexity of Crosses and Hybrids.—There are several possible ways of indicating the complexity of a cross. For example, when we cross yellow round by green wrinkled, we are dealing with two pairs of alleles at the same time (green and its normal allele and wrinkled and its normal allele). We could describe this cross by saying that it involves two pairs of alleles, or that it involves two mutations and their normal alleles, or simply two mutations ("and their normal alleles" being understood). We can indicate the complexity of the F_1 offspring produced by a cross in various ways. Thus when yellow round is crossed to green wrinkled, we could say (1) that the F_1 is hybrid for two pairs of alleles, or (2) that it is hybrid at two loci, or (3) that it is hybrid for two mutations, or (4) that it is a dihybrid.

The Abbreviated Genotypic Ratio.—It will be recalled that when the yellow round race of peas is crossed to the green wrinkled $\left(\dfrac{+\ +}{+\ +} \times \dfrac{g\ w}{g\ w}\right)$, the phenotypic ratio in the F_2 is 9 yellow round : 3 yellow wrinkled : 3 green round : 1 green wrinkled. But we can

also express this ratio in terms of genes. The yellow offspring are
either $+/+$ or $+/g$, and therefore we can let one $+$ stand for
either of these two combinations, since $+$ is dominant and deter-
mines the appearance of the offspring. Likewise we can let one $+$
(in the wrinkled locus) stand for either $+/+$ or $+/w$. One g can
stand for g/g, and one w for w/w. The nine yellow round could
then be represented as $9 + +$, the three yellow wrinkled as $3
+ w$, the three green round as $3\ g +$, and the one green wrinkled
as $1\ g\ w$. That is, the F_2 ratio, expressed briefly in terms of genes
is $9 + + : 3 + w : 3\ g + : 1\ g\ w$. We can refer to this as the
$9 : 3 : 3 : 1$ *abbreviated* genotypic ratio. In this ratio the first class
$(9 + +)$ contains both dominants, the second class $(3 + w)$ con-
tains one dominant, the third class $(3\ g +)$ contains the other
dominant, and the fourth class $(1\ g\ w)$ contains neither dominant.
Thus, if we let A and B stand for dominant genes and a and b for
their recessive alleles, the abbreviated genotypic ratio is $9\ AB : 3
Ab : 3\ aB : 1\ ab$.

**A Cross Involving Two Pairs of Alleles Influencing the
Same Trait (Combs in
Chickens).**—When Mendel
crossed yellow round peas by
green wrinkled, he was dealing
with two pairs of genes each in-
fluencing a different trait. One
pair was concerned with seed
color (whether yellow or green);
the other pair with seed texture
(whether round or wrinkled).
But any trait is due to the in-
teraction of a great many genes,
and a cross might involve two
or more pairs of genes influenc-
ing the *same* trait. One of the
earliest crosses of this kind,
made by the English geneticists
Bateson and Punnett, dealt
with the combs of chickens.

Fig. 30. Various types of combs in
chickens. (From Bateson's *Mendel's
Principles of Heredity*, by permission
of G. P. Putnam's Sons.)

There are various races of
chickens with different kinds of combs. The most common type
of comb seems to be one known as *single* (Fig. 30). We shall

regard this as the normal comb (since the normal is usually the most common). Other types of comb besides single are *pea, rose,* and *walnut.* Pea and rose are due to dominant mutations in separate chromosomes. We can indicate the mutant genes by P (pea) and R (rose), and their normal alleles by $+$ signs. Single is
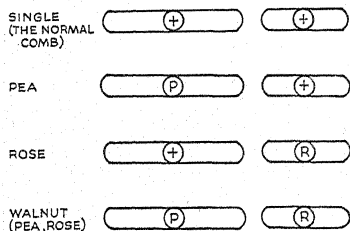
FIG. 31. Chromosome diagrams of chickens, showing the genes for combs (in the gametes).

therefore $+ +$, pea is $P +$, and rose $+ R$ (Fig. 31). Walnut is a combination of the two mutations, or PR. These are the gametes of the pure races.

When walnut is crossed to single, PR and $+ +$ combine and produce F_1 of genotype $\frac{P\ R}{+\ +}$. Since each mutated gene is dominant to its normal allele, the F_1 are walnut (this type of comb being due to the combination of pea and rose). When the F_1 are inbred the F_2 abbreviated genotypic ratio is 9 PR : 3 $P +$: 3 $+ R$: 1 $+ +$. This follows from the fact that P and R are the dominant genes, so that the first class (9 $P R$) contains both dominants, the second class (3 $P +$) one dominant, the third class (3 $+ R$) the other dominant, and the fourth class ($+ +$) neither dominant. The offspring represented by the first item of the above ratio (9 $P R$) are walnut, since they are a combination of pea and rose. Those represented by the second item (3 $P +$) are pea; by the third (3 $+ R$), rose; and by the fourth (1 $+ +$), single. The F_2 phenotypic ratio is therefore 9 walnut (PR) : 3 pea ($P +$) : 3 rose ($+ R$) : 1 single ($+ +$).

In the above case we crossed walnut by a single, but we might have got the same results by crossing a pea by a rose $\left(\frac{P +}{P +} \times \frac{+ R}{+ R}\right)$. The F_1 from this cross are $\frac{P +}{+ R}$ (walnut), and they are of the same genotype as $\frac{P\ R}{+\ +}$, or the F_1 from single $\times$ walnut $\left(\frac{+ +}{+ +} \times \frac{P\ R}{P\ R}\right)$. Hence the F_2 are the same.

The 9 : 7 Ratio.—In sweet peas the flowers are normally purple, but there are two white-flowered varieties which we can refer

to as white race a and white race b. Each white race is due to a recessive mutation, and the two mutations are in separate chromosomes. We can indicate the mutant genes by a and b, and their normal alleles by $+$ signs. The gametes of the pure races are therefore $+\,+$ (purple), $a\,+$ (white race a), $+\,b$ (white race b). It is possible to get a third white race which combines both mutant genes (ab).

It is not surprising that the two mutations a and b should have the same effect when it is remembered that each simply interferes entirely with pigment development. This is comparable to ruining a watch by destroying either the spring or one of the cog-wheels—operations on totally different parts. The two normal alleles (the $+$'s) are both necessary for purple flowers, and they are therefore referred to as *complementary genes*.

A cross of the two white races $\left(\dfrac{a\,+}{a\,+} \times \dfrac{+\,b}{+\,b}\right)$ produces F_1 of genotype $\dfrac{a\,+}{+\,b}$. This is purple, because the $+$ alleles are dominant.

When the F_1 are inbred they produce F_2 in the $9 : 3 : 3 : 1$ ratio, in which the first class as usual contains both dominants $(+\,+)$, the second one dominant $(+\,b)$, the third the other dominant $(a\,+)$, and the fourth neither dominant $(a\,b)$. Hence the F_2 ratio is $\cdot 9\,+\,+$ (purple) : $3\,+\,b$ (white) : $3\,a\,+$ (white) : $1\,a\,b$ (white). Since the last three classes are white, the F_2 phenotypic ratio is 9 purple : 7 white. This is obviously a *modified* $9 : 3 : 3 : 1$ ratio.

The 9 : 3 : 4 Ratio.—Bateson worked out another modified $9 : 3 : 3 : 1$ ratio in sweet peas. Here red flowers are due to a recessive mutation (purple being normal). We can designate the mutant gene as r, and its normal allele as $+$. The mutant gene r is in a separate chromosome from either of the two white mutations (a and b). We might now cross one of the white races, say a, to the red. The genotypes of the parents would be $\dfrac{a\,+}{a\,+}$ (white) $\times$ $\dfrac{+\,r}{+\,r}$ (red). The F_1 would be $\dfrac{a\,+}{+\,r}$ (purple). When the F_1 were inbred they would produce F_2 in the $9 : 3 : 3 : 1$ ratio, and as usual the first class would contain both dominants $(+\,+)$, the second class one dominant $(+\,r)$, and so on. The last class would be pure for both recessives $(a\,r)$. It would appear white, because any plant

pure for a cannot form pigment, regardless of what other genes it might have. Therefore, when the F_1 are inbred, they produce F_2 in the ratio of $9 + +$ (purple) : $3 + r$ (red) : $3\ a +$ (white) : 1 $a\ r$ (white), or 9 purple : 3 red : 4 white.

The 13 : 3 Ratio.—Chickens illustrate still another modified 9 : 3 : 3 : 1 ratio, worked out by Bateson and Punnett. In chickens colored feathers are normal. There are at least two mutant genes which cause white. One is found in white Leghorns and is *dominant* to its normal allele. Another is found in other chickens and is *recessive* to its normal allele. We can use the symbol A for the dominant white and b for the recessive white. The two mutant genes are in different chromosomes. The gametes of the pure races are $+ +$ (normal or colored), $A +$ (dominant white), $+ b$ (recessive white). It is possible to get a race which combines both mutant genes ($A\ b$). This is also white. A cross of the two simple white races $\left(\dfrac{A\ +}{A\ +} \times \dfrac{+\ b}{+\ b}\right)$ gives F_1 of genotype $\dfrac{A\ +}{+\ b}$. These are white because A is dominant. When the F_1 are inbred they produce F_2 in the usual 9 : 3 : 3 : 1 ratio. The first class contains both dominants ($9\ A +$), the second contains one dominant ($3\ A\ b$), the third contains the other dominant ($3 + +$), and the fourth neither ($1 + b$). Thus the F_2 ratio is $9\ A +$ (white) : $3\ Ab$ (white) : $3 + +$ (colored) : $1 + b$ (white), or 13 white : 3 colored.

The 15 : 1 Ratio.—Just another example of a modified 9 : 3 : 3 : 1 ratio, worked out by G. H. Shull, might be mentioned. In the wild plant known as shepherd's purse the seed case or capsule is normally triangular in shape. But there is also a race with round capsules produced by the combination of two mutations each in a different chromosome, and each recessive to its normal allele. We can designate the mutated genes by a and b, and their normal alleles by $+$ signs. Neither mutation by itself has any influence on the shape of the capsule, even when the plant is pure for it. A cross of the two races $\left(\dfrac{+\ +}{+\ +} \times \dfrac{a\ b}{a\ b}\right)$ gives F_1 of genotype $\dfrac{a\ b}{+\ +}$ (with normal triangular capsules). These inbred give F_2 in the ratio of $9 + + : 3 + b : 3\ a + : 1\ ab$. Only the last class ($1\ ab$) is pure for both a and b and therefore it alone has round capsules, the rest having triangular capsules. Thus we get in the F_2 a ratio of 15 triangular : 1 round.

Crosses Involving Several Interacting Genes (Influencing Coat Color in Mice).—Heredity may become very complex when a cross involves several pairs of alleles which influence the same trait. Cases of this sort have been disentangled in some of the lower animals, especially rodents. In mice mutations have taken place in about a dozen genes that enter into the production of coat color. The normal coat color of mice is gray (sometimes referred to as "agouti"). Mutations (mostly recessive) have changed the normal coat color to other colors known as albino, black, cinnamon, dilute, and so forth. If a simple mutant race is crossed to the normal gray race the offspring are hybrid at just one locus, and when they inbreed they produce F_2 offspring in the 3 : 1 ratio. Thus a gray by albino gives 3 gray : 1 albino in the F_2; gray by black gives 3 gray : 1 black in the F_2. But the albino gene is the allele of the normal gene from which it arose, and the black gene the allele of another normal gene from which *it* arose. The two normal genes are at different loci and in different chromosomes. We could represent the normal race by $\dfrac{+\ +}{+\ +}$, the albino race by $\dfrac{a\ +}{a\ +}$, and the black race by $\dfrac{+\ b}{+\ b}$ (where the $+$'s are normal genes for coat color, a the recessive mutated gene for albino color, and b the recessive for black). By crossing an albino by a black $\left(\dfrac{a\ +}{a\ +} \times \dfrac{+\ b}{+\ b}\right)$ it would be possible to get in the F_2 some offspring of genotype $\dfrac{a\ b}{a\ b}$; that is, offspring pure for both albino and black. These would appear albino, the same as the simple albino $\left(\dfrac{a\ +}{a\ +}\right)$ because in an animal pure for albino, no color genes can express themselves. The animal simply lacks pigment.

It would be possible to cross a black-carrying albino to some other mutant, say, "cinnamon." The black-carrying albino has two of the mutant genes, a and b, in two of its chromosomes, and the normal allele of cinnamon in the third, thus: $\dfrac{a\ b\ +}{a\ b\ +}$. The cinnamon mouse has a mutated gene in the third chromosome but is otherwise normal, thus: $\dfrac{+\ +\ c}{+\ +\ c}$ (where c stands for cinnamon, a reces-

sive). Thus a cross of the black-carrying albino and the cinnamon is $\frac{a}{a}\frac{b}{b}\frac{+}{+} \times \frac{+}{+}\frac{+}{+}\frac{c}{c}$. The F_1 are $\frac{a}{+}\frac{b}{+}\frac{+}{c}$ (where $a\ b\ +$ came from the black-carrying albino parent and $+ + c$ from the cinnamon). Thus the F_1 are hybrid at three loci all influencing *one* trait (coat color). In appearance they are gray (the normal alleles being dominant).

If two such hybrids should interbreed a highly varied lot of off-spring would be produced, but we could easily derive these. We
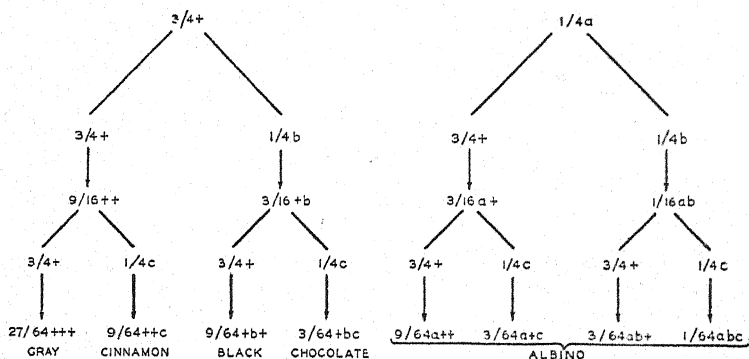
FIG. 32. The derivation of the offspring of mice of genotype $\frac{a}{+}\frac{b}{+}\frac{c}{+}$.

should first put down the abbreviated F_2 genotypic ratio when the parents are hybrid at just the a locus ($a/+$). This is $3 + : 1\ a$, or if expressed in fractions $\frac{3}{4} + : \frac{1}{4}\ a$ (Fig. 32). We should next subdivide each of these classes into $\frac{3}{4} +$ and $\frac{1}{4}\ b$ on the basis of the b locus. The combination of the two ratios (for loci a and b) would be $\frac{9}{16} + + : \frac{3}{16} + b : \frac{3}{16}\ a + : \frac{1}{16}\ ab$. This is the usual di-hybrid ratio. Each of the above four classes could next be sub-divided into $\frac{3}{4} +$ and $\frac{1}{4}\ c$, giving the eight classes shown in Fig. 32 (bottom part). Next we should work out the appearance of these eight classes. Thus the first class $(+ + +)$ would be gray because it has all three normal alleles. The second class $(+ + c)$ would be cinnamon because it is pure for c and has the normal alleles at the other two loci. One class would be $+ b\ c$ (with the normal allele at the first locus and pure for the black and cinnamon alleles at the other two loci). This class would be black-cinnamon, a combination that results in a coat-color known as chocolate.

All classes pure for *a* would be albino regardless of what they carried at the other loci.

Although the above F_2 results are complicated, yet fundamentally they conform to Mendel's principle. The alleles at each locus undergo segregation without previous admixture. The same sort of thing holds true of eye color in man, though the number of mutations involved and their exact effects have not been worked out.

Reversion.—We saw that in sweet peas there were two white races, each due to a different recessive mutation $\left(\dfrac{a\ +}{a\ +}\ \text{and}\ \dfrac{+\ b}{+\ b}\right)$.

When the two are crossed the offspring are purple $\left(\dfrac{a\ +}{+\ b}\right)$, because each white carries the normal gene which the other lacks. If we had not known that the two whites were due to different mutations, we might have thought that they belonged to the same race, and it would have been difficult to see why they should produce purple when interbred.

Before the days of Mendel it had been noticed by breeders that a mutant type after having bred true for many generations sometimes produced offspring that resembled the ancestral type (throwbacks). They referred to this return to the ancestral type as "reversion" or "atavism." It is now known that reversions are often to be explained essentially in the same manner as are the purples thrown by whites in sweet peas.

Nomenclature.—A word or two might be said at this point about the naming of genes. It makes very little difference what names and symbols we use in designating genes as long as our nomenclature is clear and convenient. We usually name a mutated gene after the appearance of the mutant animal or plant. Thus if a mutation changed a pea plant from tall to dwarf, we should call the mutated gene dwarf and designate it by the symbol *d*. A small letter tells us the mutated gene is recessive and a large letter that it is dominant.

As for the labeling of normal genes, in this book we have simply been using $+$ signs for normal alleles, the position of the $+$ in a formula indicating which normal allele a given $+$ represents. But some authors would use $+^d$, for example, to indicate the normal allele of dwarf; others would use d^+ for the same thing. Still again

some would use D for the normal allele of d; that is, the same letter for both alleles but a large one for the dominant allele and a small one for the recessive. However, this method of designating the alleles has the disadvantage that D as well as d suggests dwarf.

An older method of naming genes is bound up with the theory that a dominant trait is due to the presence of a gene, a recessive to its absence (the "presence and absence" theory). Thus in peas purple flower color is supposed to be due to three genes, one for color in general (C), another for red (R) and a third for blue (B). Red is supposed to be due to the loss of blue, and white to the loss of either "color" (C) or red (R). The absence of blue (B) is indicated by b, so that red would be CRb and a purple plant hybrid for red would be $\dfrac{C\ R\ b\ \text{(red)}}{C\ R\ B\ \text{(purple)}}$. In a pure purple $\left(\dfrac{C\ R\ B}{C\ R\ B}\right)$ blue is allelic to itself but in the hybrid $\left(\dfrac{C\ R\ b}{C\ R\ B}\right)$ it would be allelic to its "absence," and in general a gene is supposed to be allelic either to itself or to its absence.

It is possible that a purple pea would be changed to red if it lost blue pigment, but it does not follow that the production of blue by the normal plant is due to just one gene for blue. Rather, it is due probably to the interaction of many genes. Hence we cannot name any one in particular *the* gene for blue. The same sort of thing applies to red. Moreover, the normal alleles of the two white mutations are equally important for pigmentation, since the plant cannot develop color without *both* normal alleles, and it is therefore arbitrary to label one "color" (C) and the other red (R). Finally it is much simpler to name mutant genes after the appearance of the mutant than to name normal genes after their supposed effects. For all these reasons the "presence and absence" nomenclature is not desirable. But in many organisms, as sweet peas and mice, genes were originally named on the basis of the "presence and absence" theory, and some authors prefer to retain the original names.

Proof That Genes Exist.—Mendel clearly demonstrated by his crosses that the hereditary material of a plant or animal consists of separable units—the units that we now call genes. Take for example the cross of yellow round and green wrinkled. Before Mendel made this cross it would have been possible to maintain

that the difference between the yellow round race and the green wrinkled was due to just one difference in their hereditary materials—that the yellow round race had one kind of hereditary material, and the green wrinkled another kind, and that each kind was just one substance which was not separable into parts. In this case the plant formed by crossing the two races would have been hybrid for just one pair of substances, not for two pairs. It would accordingly have formed just two classes of sperm and egg cells, one class with the yellow round substance, the other with the green wrinkled. This in turn would have meant that the hybrid could have formed only two classes of offspring—yellow round and green wrinkled. But Mendel found the hybrid could also form the recombinations yellow wrinkled and green round. This showed that yellow was *separable* from round, and green from wrinkled.

In brief, Mendel showed that it was possible to separate from each other traits that were previously together in the same race. This he did by bringing together traits (in the F_2) that were previously in separate races (in the P_1). Thus he showed that the hereditary material or germ plasm of the plant consisted of separable units. These we now call genes.

Mendel and the Chromosome Theory of Heredity.—It is important now that we should understand the relation of Mendel's work to chromosomes. In Mendel's day it was not yet known that the genes were carried by the chromosomes. In fact chromosomes themselves were practically unknown as objects observable under the microscope. It was only later (in the fourth quarter of the last century) that chromosomes were carefully observed by microscopic workers, especially van Beneden, Strassburger, and Flemming. It was seen that chromosomes ran in pairs and that members of a pair separate or segregate from each other at the reduction division. Since Mendel's experiments had previously shown that alleles do the same thing, it at once became apparent (after the rediscovery of Mendel's principle) that alleles and chromosomes go together in their transmission to the reproductive cells and to the offspring, and the conclusion was drawn that the genes are in the chromosomes.

Moreover, Mendel showed that two pairs of alleles were assorted independently of one another. This was also seen to apply to the chromosomes on general grounds. For two chromosomes belonging to two different pairs are not tied together and hence do not

necessarily have to line up on the same side of the mid-line when the reduction division takes place. They might equally well line up on opposite sides. Thus two pairs of chromosomes show independent assortment, just as do two pairs of alleles in Mendel's hybrids.

In brief, a *parallelism* was early observed in the distribution of chromosomes and genes in heredity, as shown by the fact that (1) both chromosomes and genes run in pairs, (2) the members of a pair (whether chromosomes or genes) separate or segregate at the reduction division, and (3) the members of different pairs (again whether chromosomes or genes) are assorted independently of one another. This parallelism led to the conclusion that the chromosomes contain the genes. The theory that the hereditary units or genes are contained in the chromosomes is known as the *chromosome theory*.

The chromosome theory was arrived at shortly after the rediscovery of Mendel's principle in 1900. Boveri and Sutton in 1904 were among the first to point to the parallelism in the behavior of chromosomes and genes and to suggest that the chromosomes contained the genes. At about the same time E. B. Wilson advocated the chromosome theory very strongly on the basis of the same general evidence. In 1906 the theory was clearly summarized in a book by R. H. Lock, *Recent Progress in the Study of Variation, Heredity and Evolution.* In 1911 and later, Morgan in collaboration with Muller, Bridges, and Sturtevant gave further strong experimental evidence in support of the chromosome theory, leading to its final acceptance by geneticists in general.

It should be emphasized that no one man is to be credited with the chromosome theory. The situation is very much the same as in the development of the atomic theory of physics. Just as that theory was the result of the growth of the science of physics, so the chromosome theory was the result of the growth of biology. But the name most prominently associated with the development of the theory must be that of Mendel. For it was he who showed how the genes were distributed in heredity, and when the chromosomes were later seen to be distributed in the same way, biologists were led to the conclusion that the genes were contained in the chromosomes. Thus Mendel's experiments laid the foundation of the chromosome theory.

SUMMARY

1. At the metaphase stage in the reduction division, two different kinds of chromosomes (A and B) or their homologues (a and b) might line up either on the same side of the mid-line $\left(\dfrac{A\ B}{a\ b}\right)$ or on opposite sides $\left(\dfrac{A\ b}{a\ B}\right)$. This is due to the fact that A is not tied to B (nor a to b). Hence A and B (or a and b) might go either to the same pole or to opposite poles, with equal likelihood. This is known as independent assortment.

2. Genes in different chromosome pairs are assorted independently of each other because the chromosomes themselves are.

3. When a pea with yellow round seeds is crossed to a green wrinkled pea $\left(\dfrac{+\ +}{+\ +} \times \dfrac{g\ w}{g\ w}\right)$, the F_1 is hybrid for two pairs of genes in separate chromosome pairs $\left(\dfrac{g\ w}{+\ +}\right)$. At metaphase in the reduction division, g and w (or their normal alleles) might line up either on the same side of the mid-line, or on opposite sides $\left(\dfrac{g\ w}{+\ +} \text{ or } \dfrac{g\ +}{+\ w}\right)$, with the result that the hybrid produces four classes of sperm and egg cells: $g\,w$ and $+\,+$ from the first line-up, and $g\,+$ and $+\,w$ from the second (or $+\,+$, $+\,w$; $g\,+$, $g\,w$).

4. The above four classes of gametes might have been derived by first putting down the two classes for the g-locus ($\frac{1}{2}\,+$ and $\frac{1}{2}\,g$), then subdividing each of these into the two classes for the w-locus ($\frac{1}{2}\,+$ and $\frac{1}{2}\,w$), and then combining the fractions, thus:

$$
\begin{array}{ccccccc}
& \frac{1}{2}\,+ & & & & \frac{1}{2}\,g & \\
\swarrow & & \searrow & & \swarrow & & \searrow \\
\frac{1}{2}\,+ & & \frac{1}{2}\,w & & \frac{1}{2}\,+ & & \frac{1}{2}\,w \\
\downarrow & & \downarrow & & \downarrow & & \downarrow \\
\frac{1}{4}\,+\,+ & & \frac{1}{4}\,+\,w & & \frac{1}{4}\,g\,+ & & \frac{1}{4}\,g\,w
\end{array}
$$
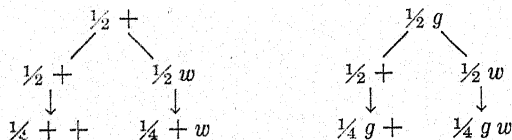
5. When the above di-hybrid is self-fertilized, each of the four classes of sperm cells combines with each of the four classes of egg cells (as shown by the "checkerboard" method). The resulting 16 combinations produce F_2 offspring in the ratio of 9 yellow round : 3 yellow wrinkled : 3 green round : 1 green wrinkled.

6. The above 9 : 3 : 3 : 1 ratio can be derived by combining the simple ratios given by each gene pair separately. Thus the F_2 ratio for the yellow-green pair is 3 yellow : 1 green, or $\frac{3}{4}$ yellow : $\frac{1}{4}$ green. Each of these classes is subdivided into $\frac{3}{4}$ round and $\frac{1}{4}$ wrinkled, and the fractions are then combined, thus:

$$\text{¾ yellow} \qquad\qquad\qquad \text{¼ green}$$

¾ round   ¼ wrinkled   ¾ round   ¼ wrinkled
↓   ↓   ↓   ↓

$\frac{9}{16}$ yellow round $\frac{3}{16}$ yellow wrinkled $\frac{3}{16}$ green round $\frac{1}{16}$ green wrinkled
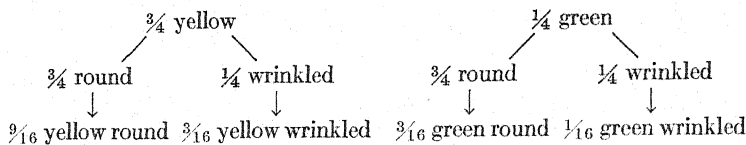
7. When a yellow round tall pea is crossed to a green wrinkled dwarf pea $\left(\dfrac{+\ +\ +}{+\ +\ +} \times \dfrac{g\ w\ d}{g\ w\ d}\right)$, the F_1 is a tri-hybrid $\left(\dfrac{g\ w\ d}{+\ +\ +}\right)$. At the metaphase in the reduction division the first two chromosome pairs line up in two possible ways, as before $\left(\dfrac{g\ w}{+\ +} \text{ or } \dfrac{g\ +}{+\ w}\right)$ and d (or its normal allele) might be on either side of the mid-line in each case $\left(\dfrac{g\ w\ d}{+\ +\ +} \text{ or } \dfrac{g\ w\ +}{+\ +\ d}\right.$;

$\dfrac{g\ +\ d}{+\ w\ +} \text{ or } \left. \dfrac{g\ +\ +}{+\ w\ d}\right)$. As a result the tri-hybrid produces eight classes of gametes in equal proportions: $g\ w\ d$, $+\ +\ +$ from the first line-up; $g\ w\ +$ and $+\ +\ d$ from the second; and so on. These eight classes might also have been derived by a continuation of the branching process shown in paragraph 4, each of the four classes of gametes therein derived being subdivided into $\frac{1}{2}\ +$ and $\frac{1}{2}\ d$.

8. When the tri-hybrid is self-fertilized, each of the eight classes of sperm cells can combine with each of the eight classes of eggs, giving 64 possible combinations from which the F_2 are derived (as can be shown by the "checkerboard" method).

9. The F_2 produced by the above tri-hybrid $\left(\dfrac{g\ w\ d}{+\ +\ +}\right)$ can be derived directly by first putting down the proportions of the F_2 classes produced by the first two pairs of genes (shown in paragraph 6), then subdividing each of these classes into ¾ tall and ¼ dwarf, and then combining the fractions, as shown below, for the first subdivision.

$$\frac{9}{16} \text{ yellow round}$$

¾ tall   ¼ dwarf
↓   ↓

$\frac{27}{64}$ yellow round tall $\frac{9}{64}$ yellow round dwarf
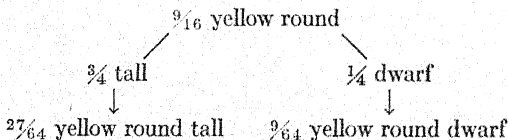
10. In all of Mendel's di-hybrids, each mutant gene influenced a different trait. But two mutations might influence the same trait, and if they are in different chromosomes, they undergo independent assortment. Hence F_1 that are hybrid for two such mutations produce F_2 in the usual $9 : 3 : 3 : 1$ ratio if the F_2 are classified according to their genes, but

often two or more such F_2 classes have the same appearance, and the F_2 phenotypic ratio is then modified accordingly.

11. Mendel proved that the germ plasm consisted of separable units which we now refer to as "genes." He did this by showing that two mutations might be assorted independently in the di-hybrid. The two mutant genes (and their two normal alleles) must therefore be separable units.

PROBLEMS

1. In the garden pea tall ($+$) is dominant to dwarf (d) and red flower color ($+$) to white (w). A pure tall red is crossed to a dwarf white. Give the P_1 phenotypes and genotypes, the gametes of the P_1, the F_1 phenotype and genotype, the gametes of the F_1 and the F_2 phenotypic ratio. Tell how this F_2 ratio would be derived by (a) the indirect method, (b) the direct method.

2. A pure tall white pea is crossed to a pure dwarf red. Give the P_1 phenotypes and genotypes, the F_1 phenotype and genotype, and the F_1 gametes. Tell why the F_2 from this cross would not differ from the F_2 in question 1.

3. Given peas of the genotypes designated below. Derive the phenotypic ratios in which the offspring are produced. (In this problem and those that follow, derive ratios by the direct method, unless otherwise stated.)

$$\textbf{a. } \frac{d\ \ w}{+\ +} \times \frac{d\ w}{d\ \ w} \qquad \textbf{c. } \frac{d\ w}{d\ +} \times \frac{d\ w}{d\ \ w}$$

$$\textbf{b. } \frac{d\ \ w}{+\ +} \times \frac{d\ \ w}{+\ w} \qquad \textbf{d. } \frac{d\ +}{+\ +} \times \frac{+\ w}{+\ w}$$

4. A cross of a tall red pea to a dwarf white produces the following count of offspring: 58 tall red, 49 dwarf red. Give the genotypes of the parents. *Hint:* in problems of this type first put down the phenotypes of the parents, each followed by horizontal lines for the gene pairs, thus: tall red $\left(\dfrac{-\ -}{}\right) \times$ dwarf white $\left(\dfrac{-\ -}{}\right)$. Then fill in as many genes as you can from inspection of the parents themselves, thus: tall red $\left(\dfrac{-\ -}{+\ +}\right) \times$ dwarf white $\left(\dfrac{d\ w}{d\ w}\right)$. Then inspect the offspring and fill in the remaining genes, but consider each trait separately in so doing.

5. A tall red by a dwarf red produces a dwarf white offspring. Give the genotypes of the parents.

6. In man brown eyes ($+$) are dominant to blue (b) and dark hair ($+$) to red (r). A man has brown eyes and red hair. He marries a woman with

blue eyes and dark hair. They have a child with brown eyes and red hair and one with blue eyes and dark hair. Give the genotypes of the parents and the children.

7. In horses black (B) is dominant to chestnut $(+)$ and trotting $(+)$, a gait in which the legs move in pairs diagonally but not quite simultaneously, is dominant to pacing (p), in which the legs move in lateral pairs. A black pacer is bred to a chestnut trotter and the resulting colt is a chestnut pacer. Give the genotypes of the parents and the offspring.

8. In snapdragons tall $(+)$ is dominant to dwarf (d) and red flowers $(+)$ are incompletely dominant to white (w), the hybrid being pink. A pure tall white is crossed to a pure dwarf red, and the F_1 are self-fertilized. Give the F_2 phenotypic ratio.

9. In snapdragons broad leaves are incompletely dominant to narrow, the hybrid leaves being of intermediate width. A broad red plant is crossed to a narrow white. Give the genotypes and phenotypes of the F_1 and the F_2 phenotypic ratio if the F_1 are self-fertilized.

10. The normal Drosophila has gray body, red eyes, and straight wings. Black body (b), pink eyes (p) and bent wings (bt) are three recessive mutations, each in a separate chromosome, the normal allele of each being designated by $+$.

A pure gray red long fly is crossed to a black pink bent, and the F_1 are interbred. Give the P_1 phenotypes and genotypes, the P_1 gametes, the F_1 genotype and phenotype. Derive the F_1 classes of gametes by the "branching" method. Derive the F_2 abbreviated genotypic ratio by the branching method, and below each abbreviated genotype put its phenotype.

11. In the cross of a pure gray red long fly by a black pink bent, what fraction of the F_2 are gray pink straight? Why? What fraction of the F_2 are gray pink straight of genotype $\dfrac{b}{+}\dfrac{p}{p}\dfrac{bt}{+}$?

12. A chicken pure for pea comb is crossed to a pure rose, and the F_1 is crossed to a single. Give the phenotypes and genotypes of the P_1, the genotype and phenotype of the F_1, and the genotypic and phenotypic ratio in the F_2.

13. Give four possible genotypes of a walnut chicken. If a pure walnut were crossed to a single, and the F_1 interbred, in what ratio would walnuts of the four possible genotypes be produced?

14. Four walnut hens are crossed to single, and each produces a large number of chicks. The first produces only walnuts, the second produces only walnuts and peas, the third only walnuts and rose, and included among those produced by the fourth are singles. Give the genotypes of each of the four hens under discussion.

15. Suppose we were given one of the F_2 walnuts from a cross of pure walnut by single, and asked to determine its genotype. How might we do this?

16. In corn the seeds are normally purple, due to pigment in the "aleurone" layer of the seed (the layer of cells immediately below the seed coat). Two recessive mutations in separate chromosomes changed purple aleurone to white. In the nomenclature of the "presence and absence" theory the two normal alleles of the mutant genes are designated as CR, meaning the gene for color and the gene for red; and the two white genes are designated as c and r, meaning the absence of C and of R. Let us instead designate the white genes as w_1 and w_2 and the normal allele of each as $+$.

Two plants, each of which grew from a seed with white aleurone, are crossed to each other, and they produce F_1 seeds (on their cobs) all with purple aleurone. Give the phenotypes and genotypes of the P_1, the gametes of the P_1, the genotype and phenotype of the F_1, and the gametes of the F_1. Give the abbreviated genotypic ratio in the F_2 (the F_1 being inbred) and below each abbreviated genotype put its phenotype.

17. Give the phenotypic ratio in which the offspring are produced if a purple aleurone corn plant of genotype $\dfrac{w_1\ w_2}{+\ +}$ is crossed to a plant of genotype (1) $\dfrac{w_1\ w_2}{w_1\ w_2}$, (2) $\dfrac{w_1\ +}{w_1\ +}$, (3) $\dfrac{+\ w_2}{+\ w_2}$.

18. Two corn plants are crossed. One grew from a seed with purple aleurone, the other from a seed with white aleurone. The offspring seeds from the cross are produced in the ratio of 1 purple : 3 white. Give the genotypes of the parents.

19. In corn a recessive mutation changed purple aleurone to red. Designate the mutant gene as r (red) and its normal allele as $+$. A pure purple seed contains the normal alleles of $w_1\ w_2$ and r and therefore is $\dfrac{+\ +\ +}{+\ +\ +}$; a red pure for the normal alleles of w_1 and w_2 is $\dfrac{+\ +\ r}{+\ +\ r}$. Both r and its normal allele are without effect in a seed pure for either w_1 or w_2, or for both w_1 and w_2. Thus, for example, $\dfrac{w_1\ +\ +}{w_1\ +\ +}$ is white; so is $\dfrac{w_1\ +\ r}{w_1\ +\ r}$.

A plant of genotype $\dfrac{+\ +\ r}{+\ +\ r}$ (red aleurone) is crossed to one of genotype $\dfrac{w_1\ w_2\ +}{w_1\ w_2\ +}$ (white). Give the genotype and phenotype of the F_1. Let the F_1 be self-fertilized and derive the F_2 abbreviated genotypic ratio by the branching method. Below each abbreviated genotype put its phenotype.

5. MULTIPLE FACTORS

WHEN the white and black races came into contact in America, the ensuing racial intermixture seemed in no way connected with Mendel's hybridization experiments on peas. The blacks and whites crossed and produced a mixture which truly seemed like a mixing of bloods. From it neither race again emerged in its pure form, regardless of the number of generations that elapsed since the hybrids were first produced. Mendel, on the other hand, was able to extract from his crosses the races which entered the cross, as when he crossed a red- and a white-flowered race and recovered the pure red and the pure white in the F_2.

Now a red-flowered pea contains many genes for flower color and so does a white. However, the only *difference* between the two races is that white contains a mutant gene (w) and red contains the normal allele ($+$) in place of w. There is, in other words, only *one* genic difference between the two races. But between human races there is more than one genic difference. If we confine ourselves to just the genes that influence the amount of blackness in the skin of the Negro and white man, then according to studies made by C. B. Davenport the pure Negro is $\dfrac{A}{A}\dfrac{B}{B}$ and the white man $\dfrac{a}{a}\dfrac{b}{b}$. In addition to these skin color genes there are still others by which the Negro and white races differ, but they have only a minor effect on skin color and we shall omit them from our consideration. The genes A and a are in a pair of corresponding chromosomes and at corresponding loci in the Negro and white man, respectively, and are alleles. The genes B and b are also alleles but are in a different pair of chromosomes from A and a. Neither allele of a pair is dominant to the other, since the offspring of black by white are intermediate. (The size of the letter does not indicate dominance or recessiveness in the present instance, though usually it does.)

Moreover, any two genes have an equal effect on color production whether they are at the same or at different loci. Thus one black gene brings about a certain increase in darkness, two cause about twice as much, and this is true regardless of which two they happen to be. In other words, the influence of the genes on the trait is *cumulative*. Members of several gene pairs which act in a cumulative way on a trait are known as *multiple factors*.

The Negro-white Cross.—When the Negro and white races cross $\left(\dfrac{A}{A}\dfrac{B}{B} \times \dfrac{a}{a}\dfrac{b}{b}\right)$ they produce a hybrid (the mulatto) of genotype $\dfrac{a}{A}\dfrac{b}{B}$. It is true now that the F_1 mulattoes in turn produce offspring who for the most part are intermediate in color like the F_1 mulattoes themselves and who show little or no tendency to revert

FIG. 33. Skin color extremes in a mulatto family. (From Davenport, Carnegie Institution of Washington, Publication No. 188).

to the original pure racial types. But occasionally in a mulatto family there is a child who is much lighter than the rest (Fig. 33). He has, to be sure, many of the characteristics of the Negro, or rather of both the Negro and white races, and would not often be confused with a pure white child. But so far as his skin color is concerned he is almost as light as a white person. This is true even when there has been no further breeding with the white race but only the interbreeding of mulattoes of the first generation of geno-

type $\dfrac{a}{A}\dfrac{b}{B}$. Occasionally, too, a very dark child is produced, almost as black as the pure Negro.

We can readily understand the above results on the basis of Mendel's principle. The mulatto $\left(\dfrac{a}{A}\dfrac{b}{B}\right)$ is hybrid at two loci and, like any other di-hybrid, forms four classes of sperm or egg cells. These are in the present instance: $A\,B$, $A\,b$, $a\,B$, $a\,b$. When two mulattoes mate their sperm and egg cells can undergo the sixteen possible combinations usual for two di-hybrids (Fig. 34). One of the possible combinations is $\dfrac{A}{A}\dfrac{B}{B}$, produced by the union of a sperm and an egg cell each containing $A\,B$. This is the extreme dark class. Another of the combinations is $\dfrac{a}{a}\dfrac{b}{b}$, produced by the union of a sperm and egg cell each containing $a\,b$. This is the extreme light class.

There are other classes of offspring possible in addition to the two just mentioned: namely, the intermediates that connect the two extremes, and these in fact outnumber the extremes. The exact extent to which they are intermediate depends on how many genes of black and how many of white origin they have. Offspring with two black and two white are midway between the extremes. This is true regardless of which two black or white they happen to be. There are three such classes: $\dfrac{a}{A}\dfrac{b}{B}$, $\dfrac{A}{A}\dfrac{b}{b}$, $\dfrac{a}{a}\dfrac{B}{B}$. Offspring with 3 black and 1 white are darker than the strict intermediates and those with 3 white and 1 black are lighter. If we limit ourselves to the black genes we find the offspring fall into five classes: namely, those that have four black genes, those that have three, two, one, and none. The offspring vary accordingly in darkness.

We should expect to find children of all the above-mentioned five classes in a family in which the parents were F_1 mulattoes, provided the family were sufficiently large to allow for the appearance of all classes. But the extreme classes appear only in comparatively small proportions, in one out of every sixteen offspring on the average (Fig. 34). Therefore in a small family the extremes would as a rule fail to show up. On the other hand, the children

of the strictly intermediate classes (those with two black genes) are the most numerous—six out of every sixteen offspring on the average. They would often be present in a family to the exclusion of the other classes. The next most numerous classes would be those which were next darker or next lighter than the strictly
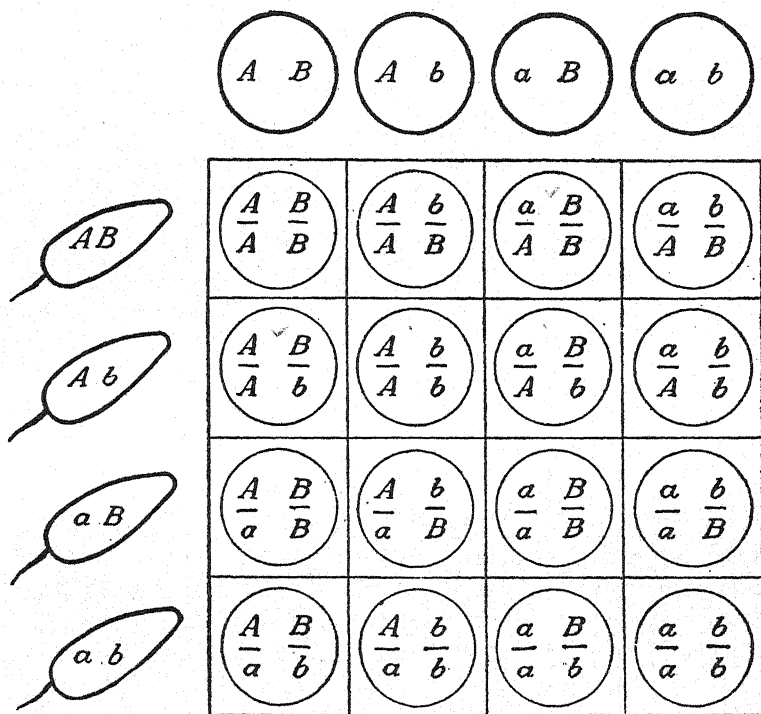
Fig. 34. The derivation of the offspring of mulattoes.

intermediate class with two black genes, and which had either three black genes or one. Each of these two classes would form four out of every sixteen offspring on the average. Finally, there would be the extreme classes which had either four black genes or none at all and which would each show up in only one out of every sixteen offspring on the average.

In brief, if the F_2 are classified according to the number of black genes they contain, the number of offspring in each class is expressed by the ratio $1:4:6:4:1$, where the first class contains

no black genes; the second, one; and so on. These facts are summarized in Fig. 35.

The Absence of Distinct Color Classes in the F_2 of the Negro-white Cross.—If we were actually to examine the children of F_1 mulattoes, we should find it practically impossible to put them into any definite color classes and to count the number in each class. We should find that the darkest offspring were connected with the lightest by all possible intermediate shades. The

Number of Black Genes	0	1	2	3	4
Relative Number of Children	1	4	6	4	1
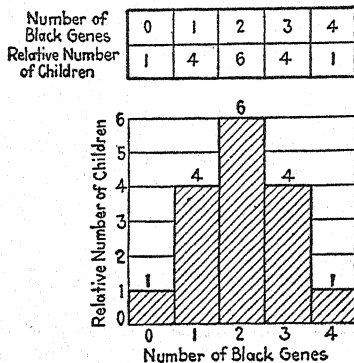
FIG. 35. The F_2 from the Negro-white cross.

offspring with a given number of black genes are not all of exactly the same color. Some are darker than others mainly because of differences in the amount of sunlight to which they have been exposed, and the darker offspring of one class sometimes connect with the lighter of the next. For example, the darkest in the class with two black genes might actually be darker than the lightest in the class with three black genes. In other words the variations in the color of classes two and three overlap. The same sort of thing is true of other neighboring classes. Thus the color distinction or "gap" between one genic class and the next is in large measure eliminated. But by the use of a special apparatus Davenport claims it was found possible to detect differences in the amount of blackness that were rather small and that would escape ordinary observation. Using this apparatus Davenport measured and compared the amount of blackness in the F_2 offspring of first generation mulattoes in Jamaica and states he was able to discover several "gaps" in the amount of their skin color (that is, shades of relatively less frequent occurrence), and so to place most of the subjects in several color classes, indicative of a $1 : 4 : 6 : 4 : 1$ ratio.

There are, however, no definite color classes apparent to the ordinary observer in a mulatto family. Instead there seems to be just one intermediate type which is subject to more or less variation in the amount of darkness. This impression is exaggerated by

the fact that the offspring who are strictly intermediate or almost so are the most abundant.

Increased Range of Variation in the F_2 as Compared to the F_1 in the Negro-white Cross.—An F_1 mulatto who had a blond white parent would tend to be slightly lighter than one who had a brunette white parent. But if we ignore slight genetic differences of this sort, the F_1 mulattoes all belong substantially to one and the same genetic class $\left(\dfrac{a\ b}{A\ B}\right)$. Hence whatever variation there is in color in the first generation is due almost entirely to differences in the amount of sunlight to which the F_1 mulattoes are exposed; that is, to *environmental* differences. But the second generation belong to several different genetic classes (five in all if classified according to the number of major black genes they contain, as shown in Fig. 35). For this reason the range of variation is considerably greater in the second generation than in the first.

If not enough offspring had been raised in the F_2 for recovery of the two extreme classes $\left(\dfrac{A\ B}{A\ B}\ \text{and}\ \dfrac{a\ b}{a\ b}\right)$, there might still have been enough children in a family to allow for the production of one containing three black genes and one white or three white genes and one black. These classes, though less dark or light than the two extreme classes, are nevertheless darker or lighter on the average than the medium class—the one with two black and two white. The F_2 would therefore still show a greater range of variation than the F_1 mulattoes, since the F_1 consists of only the one genetic class $\left(\dfrac{a\ b}{A\ B}\right)$ but the F_2 of more than one.

Proof of Mendelian Segregation in the Negro-white Cross.—The reappearance of the pure classes, or rather of the extreme skin-color classes, would not be expected even occasionally if the two races had become permanently mixed in the sense that their characteristic genes had lost their identity through mutual contamination. For otherwise the intermediate type would persist, like ink and water that had once been mixed, never to become separated again from each other. But if the genes themselves did not mix and each preserved its identity, then and only then could the F_1 mulattoes produce F_2 offspring of the extreme genetic types.

We saw that when the number of offspring considered was small, the extreme classes would often not show up in the F_2 of the Negro-white cross. But even in these cases the range of variation in skin color still is (as a rule) greater in the F_2 than in the F_1, and this in itself is evidence that the skin-color alleles segregate in the F_1 hybrids. For the increased range of variation indicates a greater number of genetic classes in the F_2 as compared with the F_1, and this in turn indicates that alleles segregate in the F_1 and undergo recombination.

In summary, then, Mendelian segregation occurs in F_1 mulattoes, as shown by (1) *the occasional appearance of the extreme color classes in the F_2* and (2) *increased range of variation in the F_2 as compared with the F_1* even when the extreme color classes do not happen to be produced.

The Fallacy of the "Blended Inheritance" Hypothesis.— The Negro-white cross is sometimes referred to as a case of "blended inheritance." It has been contrasted to Mendelian inheritance, which is sometimes referred to as "alternative inheritance," because in Mendel's experiments the offspring were either of one alternative class or the other (as red or white when the F_2 ratio was 3 red : 1 white).

It so happened that Mendel crossed races between which there was just one noticeable genic difference affecting a given trait, and the offspring of the hybrids developed one trait or the other in accordance with the type of gene that they received. But when several genic differences influence a given trait, then the difference in the trait as it appears in the F_2 is not determined by just one gene or its allele but by various combinations of several. Therefore the trait need not appear in just one of two alternative forms such as pure black or pure white. Nevertheless the individual genes are transmitted by the hybrid in the same way as when the traits themselves appear in just one alternative form or the other. In either case alleles segregate in the hybrid and the individual genes are transmitted in their pure form to the next generation. The fact that the mulatto is intermediate in appearance does not prove that his genes have also become intermediate. His color is one thing, his genes another. Mendel's law applies to genes, the ultimate units of heredity, not to traits.

It would be incorrect to state that there were two forms of inheritance, "blended" as contrasted to "alternative." Basically all

inheritance conforms to Mendel's principle—the segregation of alleles in the hybrid without previous admixture. It is only upon superficial inspection that the Negro-white cross seems to conform to some other mode of inheritance.

Other Traits Involved in the Negro-white Cross and the Complexity of the Case.—The Negro and white races as a whole never again emerge in their pure form after once they have mixed. The offspring of the F_1 mulattoes are never pure Negroes or pure Caucasians, not even the exceptional skin-color extremes. For skin color is not the only trait that is distinctive in the two races, and that depends on several genes. There are the differences in structure of their skulls, character of their hair, thickness of lips, and numerous other traits. Even if every one of these traits when considered separately appeared again in its pure form among the offspring of the mulattoes, it would be extremely unlikely that one particular child should have in pure form *all* the traits characteristic of the one race or the other. He might have the pure skin color of one of the races, say, the white, but not the typical skull structure. But *theoretically* a child that was pure Negro or pure Caucasian might turn up in the F_2 when two F_1 mulattoes mate, because all of the genes in the mulatto obey Mendel's law, regardless of what trait they influence and of how many of them there are that influence it. The genes that act as partners, *alleles*, in no instance mix with each other in the hybrid (the F_1 mulatto); they remain characteristically Negro or white and again enter separate reproductive cells before they pass on to the next generation. But that all the Negro genes should go exclusively to any one reproductive cell and all white to any other in the F_1 mulatto is much more unlikely than that two people should get two complete decks of cards after the two decks had been thoroughly shuffled and dealt out at random. That two such cells, one male and one female, should come together enormously increases the improbability of recovering the pure race.

Multiple Factors for Flower Size in Tobacco.—Numerous crosses involving multiple factors have been made in lower plants and animals. Crosses between tobacco plants might be taken in illustration.

Different races of tobacco differ as regards the length of their flowers, as determined by the length of their corollas (the part of the flower made up of petals). The flowers of one race, of course, are

not all of exactly the same length. They show all gradations in size
between two extremes (short and long), just as do men. The
medium size is the most frequent and the further a size departs
from the medium the less frequent it becomes, again just as in man.
But the average size of the two races might be distinctly different,
just as would apply to the average size of a Norwegian and of a
Japanese.

The average size is not necessarily always the same as the most
frequent, nor is the average necessarily the same as the medium
size. We might for example have a group of 100 persons made up of
two selected sizes, 10 persons 5 feet tall and 90 persons 6 feet tall.
In this group the average size of a person would be 10×5 feet $+$
90×6 feet, divided by 100, or 590/100 feet, which equals 5.9 feet.
The size that was medium between the two sizes (5 feet and 6 feet)
would be 5.5 feet. The most frequent size would be 6 feet. However,
in an ordinary population consisting of large numbers of unselected
persons, there is a continuous range of variation between extreme
small and extreme tall, with the most frequent size midway be-
tween the two. In such a population medium, average, and most
frequent sizes are about the same. Similar considerations, of course,
govern other organisms besides man.

Two races of tobacco of different average flower size were
crossed by E. M. East. We can refer to the smaller race as A, to the
larger as B. The flower size was determined by measurement of the
corollas (the corolla being the trumpet-shaped part of a flower,
consisting of the fused petals), and the measurements were made
in millimeters (a millimeter being equal to about $\frac{1}{25}$ of an inch).
In race A, 211 corollas were measured in the P_1, and the corolla
length was found to vary from about 34 mm. to about 43 mm.
(Table 1). The most frequent corolla length of race A was 40 mm.,
there being 140 corollas of this length, and this happens to be fairly
close to the average or mean length. In race B the lengths of all the
corollas measured ranged from about 88 mm. to 100 mm., and the
most frequent length was 94 mm. (again fairly close to the average
or mean length). A cross of the two races produced 173 offspring,
among which the most frequent corolla length was 64 mm., this
being about midway between the most frequent lengths of the
parents (40 mm. and 94 mm., respectively). The range in variation
among the F_1 offspring was from 55 to 70 mm. These F_1 hybrids
were inbred, and 211 of their offspring (F_2) were measured. The F_2

had about the same average size as the F_1, the most frequent length in the F_2 being 67 mm. (as compared with 64 mm. in the F_1). But *the range of variation in the F_2 was greater than in the F_1*, the size ranging from 52 to 85 mm. in the F_2 as compared with a range of 55 to 70 mm. in the F_1. This *increased range of variation in the F_2 is indicative of segregation of alleles in the F_1 hybrid.* We are here dealing therefore with another multiple factor case.

TABLE 1. EVIDENCE FOR MULTIPLE FACTORS IN TOBACCO

Corolla length	34	37	40	43	46	49	52	55	58	61	64	67	70	73	76	79	82	85	88	91	94	97	100
P_1 (Race A)	1	21	140	49																			
P_1 (Race B)																	13	45	91	19		1	
F_1								4	10	41	75	40	3										
F_2							1	5	16	23	18	62	37	25	16	4	2	2					

In the above table, class 34 includes 33, 34, 35; class 37 includes 36, 37, 38; and so on. (From E. M. East, *Genetics*, 1916.)

However, the extreme size classes are not recovered in the F_2 in the above case. This can be accounted for on the ground that not enough F_2 offspring were grown. When the F_1 is hybrid for two pairs of multiple factors, we expect each of the extreme classes in 1 out of every 16 offspring on the average in the F_2, as in the Negro-white cross. But when the F_1 is hybrid for more than two pairs of factors, the proportion of the F_2 belonging to the extreme classes is less. Thus when the F_1 is hybrid for 3 pairs we expect 1 offspring of each extreme class out of every 64 in the F_2. This is true because the F_1 hybrid for 3 pairs forms 8 classes of sperm and egg cells and these can undergo 8×8 or 64 possible combinations, only one of which results in either of the two extreme F_2 classes. These are the classes homozygous for all three pairs of factors characteristic of the one race or the other; namely, $\dfrac{A\ B\ C}{A\ B\ C}$ and $\dfrac{a\ b\ c}{a\ b\ c}$. When the F_1 is hybrid for 4 pairs of factors, it forms 16 classes of sperm and egg cells, and these can undergo 16×16 or 256 possible combinations, only one of which again results in either of the extreme classes. With 5 pairs of factors in the F_1 the number of different classes of reproductive cells is 32, and each of the extreme F_2 classes is recovered only in 1 out of every 32^2 offspring, or 1 in 1,024. With

increase in the number of factors for which the F_1 is hybrid, the proportion of the extreme classes recovered in the F_2 rapidly decreases, and it becomes necessary to grow larger and larger numbers of F_2 offspring to recover the extremes.

In the tobacco case just given 211 offspring were grown and measured in the F_2 without recovery of the extreme classes. As we should expect each of the extreme classes in 1 out of every 64 F_2 offspring on the average if the F_1 were hybrid for just three pairs of factors, the case under discussion probably involves more than just three pairs.

Other Multiple Factor Cases.—Numerous other cases could be given in illustration of multiple factors, equally as good as the above. Multiple factor differences account for differences in ear length in rabbits, length of cob in corn, and size of "hood" in hooded rats (the hood in this race being a black area on the head, neck and back on an otherwise white coat). In all instances when races are crossed differing in regard to the size of the trait mentioned and the hybrids are inbred, there is a greater range of variation in the F_2 than in the F_1.

Although in the Negro-white cross it happens that each gene has an equal effect on skin color, without dominance, we must not suppose that in cases of multiple factors the influence of the individual genes is always equal. As between two *pairs* of genes, one might have a stronger effect than another on a given trait; again, within a *single* pair one allele might be dominant to the other. If the number of gene pairs is large, the curve showing the range of variation in the F_2 is very much the same regardless of whether the influence of the individual genes is equal or somewhat unequal. It would indeed be very unusual if all the genes involved should have an exactly equal influence. Much more likely some have a greater effect than others.

Multiple Factors and Species Crosses.—In crosses involving a large number of multiple factors, the strictly intermediate class is very abundant in the F_2, as opposed to the more extreme classes. When the offspring are limited in numbers, they will in all likelihood belong to, or be close to, the strict intermediates (the median class). The F_2 upon being inbred will in turn give rise for the most part to strict intermediates.

The differences between species are often dependent on numerous multiple factors. Thus if one species is taller than another, the

difference in size is often due to a difference in numerous genes for size, and when the two species are crossed (as is possible in some cases) we do not as a rule get a 3 : 1 ratio when the F_2 are classified for size. Instead we get mostly intermediates in both the F_1 and the F_2, and in later generations as well. But often the range of variation is somewhat increased in the F_2 as compared with the F_1, and this indicates multiple factors and Mendelian segregation in species crosses.

The Hereditary Basis of "Fundamental" Traits.—How do ordinary people, those that are not "hybrids," transmit their inheritance to the offspring? And how are fundamental things like the backbone inherited? In answer to these questions it should be pointed out first of all that, strictly speaking, the backbone is not inherited. The backbone is a trait, and like all other traits, it develops; it itself is not transmitted from parent to offspring. What is transmitted are the genes upon which the development of the backbone is dependent. The fertilized egg starts its development with many different kinds of backbone genes, possibly hundreds. But practically all of those in one human being are of the same kind as in any other human being. Before a person reproduces, his mature reproductive cells receive a complete set of backbone genes (A, B, C, etc.), and when two persons mate each transmits to the child a complete set of backbone genes which is practically the same throughout from each parent. The child therefore is pure in large measure for his backbone genes $\left(\dfrac{A}{A} \dfrac{B}{B} \dfrac{C}{C} \ \cdots \right)$; so are the children of the next generation. Accordingly there are no distinct differences in the second generation, no Mendelian classes, to indicate that segregation has been taking place. Nevertheless in each generation A segregates from A, B from B, and so on.

Direct evidence of segregation would be manifest only if the original parents happened to differ with regard to their backbone genes. For only then would there be produced in the second generation of offspring the varied classes that are the visible evidence of Mendelian segregation in the F_1 hybrid. Thus we might cross two races of fishes having backbones with different numbers of vertebrae. We might then find a greater range of variation in the F_2 than in the F_1 as regards number of vertebrae in the backbone. This would tell us that the F_1 was hybrid for several pairs of alleles and that the members of each pair segregated from one

another. But in order to show that *all* the backbone genes segregate
from their alleles we should have to cross an animal that had a
backbone to one that had none. We should then breed the hybrids
and get offspring of the second generation from them. Only then
would it be possible to get in the second generation some offspring
that had backbones and others that had none, and so to demon-
strate directly that *all* the backbone genes segregated according to
the Mendelian principle.

Not any backboneless animal would serve equally well for the
above hypothetical cross. To bring out the facts clearly it should
be preferably one that had in place of the backbone some other
structure, like the gelatinous rod from which the backbone evolved,
a structure known as the notochord; and in place of the backbone
genes $(A, B, C \ . \ . \ .)$, it should have others (notochord genes
$a \ b \ c \ . \ . \ .$) to act as alleles (partners) for the backbone genes in the
hybrid $\left(\dfrac{A}{a} \dfrac{B}{b} \dfrac{C}{c} \ . \ . \ .\right)$. Needless to say, no such experiment could
actually be performed. It would be impossible at the start to
cross two animals so distantly related as one with a backbone and
one without. Even if hybrid offspring could be got from the cross it
would be necessary in turn to mate them and to get large numbers
of their offspring, enough to insure the production of the pure
parental types in the F_2. It would be necessary to raise countless
millions of offspring in order to get the rare combinations that
contained nothing but backbone genes on the one hand and noth-
ing but notochord genes on the other. Only then would it be
evident that all of the backbone genes had segregated from all of
their alleles in the hybrid and that every one of them followed
Mendel's principle.

The combinations resulting in the extreme classes would be rare
because of the large number of gene pairs that would have under-
gone independent assortment in the hybrid $\left(\dfrac{A}{a} \dfrac{B}{b} \dfrac{C}{c} \ . \ . \ .\right)$. It
would happen only in very rare instances that the hybrid would
produce a gamete having exclusively backbone genes $(A, B, C, \text{etc.})$,
and another gamete having exclusively their alleles $(a, b, c, \text{etc.})$.
On top of this it would require the accidental combination of two
gametes each of the same extreme class to produce an offspring of
pure type. Such a combination would be exceedingly rare.

That there is a large number of genes involved in the development of the backbone is highly probable. The backbone has had a long history. Its evolution has taken place over millions of years and must have involved many mutations. In this way the structure that was the precursor of the backbone gradually changed and became the backbone. But the mutated genes are the alleles of the genes from which they arose, and in a hybrid the mutated genes segregate from their alleles. This is Mendelian inheritance.

Mutations still affect the backbone genes and cause changes in the backbone—in its length, width, details of shape, etc., and in this way they give us visible evidence of the genes themselves and of their variety. Every other important trait has had a similar history. Every one is dependent in development upon a great many genes that came into existence by the process of mutation and that are still mutating occasionally. Strong evidence for this belief has been furnished by the observation of mutations in various animals and plants and in particular in Drosophila. This insect has been bred in the laboratory and kept under observation for many generations, and through mutations and breeding experiments a great many genes have become manifest in connection with each organ—the eyes, wings, and other important organs. It is highly probable that in other animals, also, every important organ is influenced by a large number of genes and is dependent in development upon them.

The Universality of Mendel's Principle.—The Mendelian principle in itself tells us nothing in particular about the inheritance of traits in man or in any other form of life; it is a general principle that applies to the gene, the unit of inheritance. The geneticist cannot tell what the hereditary basis of a trait is by mere inspection of the trait. His position is analogous to that of a person who is familiar with the principles of mechanics but who has not yet studied the engines in some particular engine room. The principles of mechanics underlie the operations of all machines, but the structure of these machines and their particular manner of operation are matters which demand separate study. Just so in biology. Mendel's principle is probably at the bottom of all inheritance (with one exception applying to plant plastids), but the precise details involved in any particular case have to be worked out. Sometimes the facts, after they have been got through hybridization, look very complicated and do not at first sight seem to be amenable to

the Mendelian principle. But in biology, as in the sciences generally, the same principle that applies to more simple events might also underlie the more complicated, even though this fact might not at first sight be apparent. It might require an extended analysis to show just how the simple principle does embrace all cases.

Mendel made a discovery which is just as fundamental in the world of living things as the discovery of atoms in the lifeless. Whether or not someone will appear in the field of biology to tell us that Mendel's principle, exact as it is, is only a close approximation to the truth, still remains to be seen. Certainly the more complicated cases of inheritance do not disprove Mendel. On the contrary they point to something dependent upon his principle: multiple factors.

SUMMARY

1. The Negro has two major genes for dark skin color $(A\ B)$, which are alleles of two for white skin $(a\ b)$. When the pure Negro and the white race interbreed $\left(\dfrac{A\ B}{A\ B} \times \dfrac{a\ b}{a\ b}\right)$, the F_1 are $\dfrac{a\ b}{A\ B}$. The F_1 produce four classes of sperm and egg cells (AB, Ab, aB, ab), and when they interbreed most of the F_2 offspring receive a combination of black and white genes and are intermediate, as $\dfrac{a\ b}{A\ B}, \dfrac{A\ b}{A\ b}, \dfrac{a\ B}{a\ B}$, etc. But occasionally a sperm and egg cell each containing AB combine, or each containing ab, and these combinations produce offspring with very dark skin $\left(\dfrac{A\ B}{A\ B}\right)$ or very light skin $\left(\dfrac{a\ b}{a\ b}\right)$.

2. If the individual genes for skin color mixed in the F_1 $\left(\dfrac{a\ b}{A\ B}\right)$, it would not be possible to recover the extreme color classes in the F_2, since all the F_2 would then be intermediate. The recovery of these classes is therefore evidence that the Negro-white cross is a case of Mendelian inheritance and not "blended" inheritance.

3. In the F_1 from the Negro-white cross, there is only one genetic class $\left(\dfrac{a\ b}{A\ B}\right)$; but in the F_2 there is more than one because of Mendelian recombination. Hence the F_2 shows an increased range in the variation of skin color, as compared to the F_1.

4. If the F_2 from the Negro-white cross are classified for the number of black genes they contain, they fall into 5 classes: 0, 1, 2, 3, 4, the first

class containing no black genes, the second, 1, etc. Each black gene causes about an equal increase in the quantity of pigment in the skin.

5. Genes which cause quantitative variation in a trait in small steps are called *multiple factors*.

6. Multiple factors are often the cause of size differences, as a difference in the corolla length in tobacco, length of cobs in corn, and length of ears in rabbits. Probably in man size differences are due largely to multiple factors.

7. When a cross involves a large number of multiple factors (say, six or more pairs) very large numbers of offspring must be reared in the F_2 for the recovery of the extreme classes, and with limited numbers, only the intermediates appear in the F_2. But nevertheless there is an increased range of variation in the F_2 as compared to the F_1, and this shows that inheritance in such cases is Mendelian.

8. Species differences are often due to multiple factors. It seems likely that there is no fundamental difference between the hereditary basis of traits that distinguish species from one another and those that distinguish variations within the same species.

PROBLEMS

1. In one family of mulattoes the parents are $\dfrac{a}{A}\dfrac{b}{B} \times \dfrac{a}{A}\dfrac{b}{B}$; in another family they are $\dfrac{A}{A}\dfrac{b}{b} \times \dfrac{a}{a}\dfrac{B}{B}$. How would the four parents compare as to skin color, assuming each "black" gene (designated by a large letter) causes an equal increase in darkness? In which of the two families would the children show more variation in skin color? Why?

2. Assign the values 4, 3, 2, 1, 0 to skin color of people having, respectively, four black genes $\left(\dfrac{A}{A}\dfrac{B}{B}\right)$, three $\left(\dfrac{a}{A}\dfrac{B}{B} \text{ or } \dfrac{A}{A}\dfrac{b}{B}\right)$, and so on, 0 being the skin color of a person of genotype $\dfrac{a}{a}\dfrac{b}{b}$ (no black genes). Give all the possible genotypes of each of the four phenotypes (4–0).

3. Give the genotypes of the parents in the following families, in which the phenotypes are enclosed in parentheses, "(2)" for example designating the skin color of a person having two black genes.

	Parents	Children
a.	(2) × (0)	1 (2) : 2 (1) : 1 (0)
b.	(2) × (0)	All (1)
c.	(2) × (2)	1 (3) : 2 (2) : 1 (1)
d.	(4) × (2)	All (3)
e.	(4) × (2)	1 (4) : 2 (3) : 1 (2)

4. Two strains of corn are of the same average size and both breed true to their size generation after generation. When the two strains are crossed, it happens that the F_1 are of the same average size as the parents, and have about the same range of variation. But when the F_1 are self-fertilized, they produce F_2 having a greater range of variation than the F_1 or the P_1, some being taller than the tallest P_1, others smaller than the smallest P_1. Explain these results, assuming that differences between only two pairs of alleles for size are involved $\left(\text{the tallest } F_2 \text{ being } \dfrac{A\ B}{A\ B}, \text{ the small-}\right.$ est $\left.\dfrac{a\ b}{a\ b}\right).$

5. Two parents are hybrid for three pairs of alleles $\left(\dfrac{a\ b\ c}{A\ B\ C}\right)$. By the branching method derive the gametes of each parent and tell in what ratio gametes of class ABC would be produced. Give the possible number of combinations between all classes of gametes of the two parents at fertilization, and tell how many of these would involve a sperm cell and egg cell both of class ABC. Tell therefore in what ratio offspring of class $\dfrac{A\ B\ C}{A\ B\ C}$ are produced by the parents in question.

6. Two parents are hybrid for four pairs of alleles $\left(\dfrac{a\ b\ c\ d}{A\ B\ C\ D} \times\right.$ $\left.\dfrac{a\ b\ c\ d}{A\ B\ C\ D}\right)$. Tell what proportion of the offspring would, on the average, be of either extreme class $\left(\dfrac{A\ B\ C\ D}{A\ B\ C\ D} \text{ or } \dfrac{a\ b\ c\ d}{a\ b\ c\ d}\right).$

7. Sometimes parents of average intelligence have a child of unusual ability (not due to unusual opportunities). If the parents were hybrid for eight pairs of multiple factors influencing intelligence $\left(\dfrac{a\ b\ c}{A\ B\ C}, \text{ etc.}\right)$, in how many children, on the average, would one of the extreme type (pure for all the genes for intelligence) be expected?

8. Assume that a tomato plant of genotype $\dfrac{a\ b\ c}{a\ b\ c}$ produces 4 oz. toma- toes and one of genotype $\dfrac{A\ B\ C}{A\ B\ C}$ produces 7 oz. tomatoes, each large letter gene causing an increase of $\frac{1}{2}$ oz. Give the weight of the tomatoes on the parent plants given below, the weight or weights of the tomatoes produced by their offspring (the F_1). Give also the lightest and heaviest tomatoes possible in later generations (if the lightest and heaviest F_1 are selected as parents).

$$\frac{A}{A}\frac{b}{b}\frac{c}{c} \times \frac{a}{a}\frac{B}{B}\frac{c}{c} \qquad\qquad \frac{a}{A}\frac{b}{B}\frac{c}{c} \times \frac{a}{a}\frac{b}{B}\frac{c}{C}$$

$$\frac{A}{A}\frac{b}{b}\frac{c}{c} \times \frac{a}{A}\frac{b}{B}\frac{c}{C} \qquad\qquad \frac{a}{A}\frac{b}{B}\frac{c}{C} \times \frac{a}{a}\frac{B}{B}\frac{c}{C}$$

9. Given tomato plants each with 5 oz. tomatoes. Plant No. 1 when self-fertilized produces F_1 with only 5 oz. tomatoes; so does plant No. 2. When 1 and 2 are crossed the F_1 tomatoes are 5 oz., but the F_2 tomatoes range in weight from 4 to 6 oz. and not beyond. Give the genotypes of plants Nos. 1 and 2. (Make the same assumptions as regards the influence of each factor for weight as in Problem 8.)

10. A 5 oz. tomato plant is self-fertilized and produces F_1 tomatoes ranging in weight from 4 to 6 oz. When this 5 oz. plant (No. 3) is crossed to plant No. 1 mentioned in the previous problem, the tomatoes in the F_1 range in weight from $4\frac{1}{2}$ to $5\frac{1}{2}$ oz. and in later generations from 4 to 7 oz. Give the genotype of the 5 oz. tomato plant in question. (See question 8 for assumed influence of genes.)

6. *THE DETERMINATION OF SEX*

ATTEMPTS of all sorts have been made artificially to cause an unborn child to be a male or a female at will. Especially have feeding experiments been tried on the mother before the birth of the child. But all such attempts have failed. One reason for their failure is that they were begun too late in development. In most instances the sex of the embryo was established long
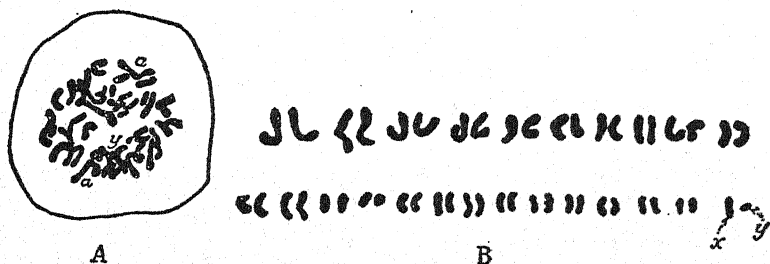
FIG. 36. The chromosomes of man, male. In *A* the chromosomes are shown as they occur in the cell; in *B* they are carefully singled out under the microscope from the cell shown in *A*. Note that each chromosome has an exact mate (as *a* and *a*), except the *x* and *y* (the last pair, lower right), the sex chromosomes. (From Painter in *Jour. of Morphology*.)

before the experiments were begun. The fact is that a person is potentially a male or a female at the moment he begins his existence, and so all attempts at determining his sex after this very earliest stage of development have been futile.

Sex Determination in Man: Female XX, Male XY.—The difference between the sexes is due to a difference in their chromosomes. It will be recalled that in the unreduced cells the chromosomes run in pairs. This applies without exception to the female. But in the male there are two chromosomes which do not exactly

match. One of these is known as the X chromosome, the other as the Y (Fig. 36). In the female there is a pair of X chromosomes instead of an XY pair. We can represent the difference between the sexes by simply showing an XX pair in the female, an XY pair in the male (Fig. 37). When the reduction division takes place in the female, all the eggs receive an X. In the male half the sperm cells receive an X and half receive a Y. The fertilized eggs therefore might be either XX or XY. All the later cells of the body produced by mitosis are like the fertilized egg and are either XX or XY, and so they develop into either a girl or a boy.

Fig. 37. Sex determination in man.

The X and the Y are referred to as the *sex chromosomes*. The remaining chromosomes, by contrast, are called *autosomes*. The process whereby offspring are caused to be either male or female is known as *sex determination*. Briefly stated, the chromosomes afford the machinery or mechanism of sex determination.

The 1 : 1 Sex Ratio.—The fertilizations that produce girls and boys are about equal, because half the sperm cells contain an X and half a Y. Of course, in a given family it does sometimes happen that there is a run of one sex or the other as might be expected in a small family. But even in large populations there is a slight departure from the expected equality in the sex ratio. Male births are somewhat in excess of female, about 105 of the one to 100 of the other in the United States. Though this difference is not so very large, it is more than would be expected on chance. It has been suggested that the Y-containing sperm cells—those that produce the males—possibly can move a little faster than their competitors and so reach the egg a little more often at the time of fertilization. The Y chromosome is smaller than the X. Perhaps it lessens the load of the sperm cell a little and makes possible slightly faster movements of Y-containing sperm as compared with X-containing.
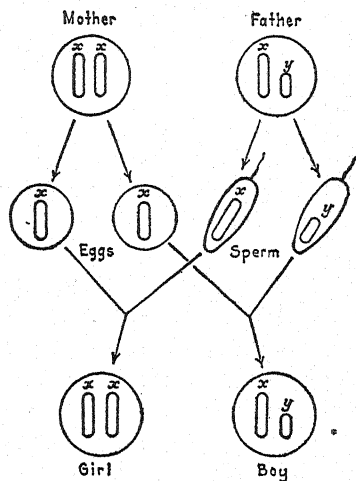
Statistics seem to show that wars increase the proportion of male births, though not to the extent usually claimed. It has been suggested that the proportion of miscarriages is somewhat decreased in war times (possibly because of the absence of husbands from home except on furlough and the resulting lessened sex relationships in the later stages of pregnancy), and as male miscarriages are more frequent than female, a lowering in miscarriages would tend to increase the proportion of male births somewhat.

Now and then we hear an account of some animal breeder who claims he has found a method of influencing the sex ratio. Undoubtedly he has tried something and found that it works apparently, but before we can accept his method as really effective we must be sure that he has found it to work in a large number of cases. Otherwise he might just be dealing with a run of one sex or the other. People are also apt to accept hearsay evidence on sex control. No well-confirmed method is really known for experimentally influencing the sex ratio in higher animals (those that belong to the same class as man—the mammals).

The Discovery of the Mechanism of Sex Determination.— The manner in which sex is determined was discovered in connection with the lower forms of life, as is so often true of biological discoveries. The X chromosome was first observed in the male of grasshoppers by McClung in 1902. McClung, however, thought that the female lacked an X; that an X caused a male, and its absence, a female. The first complete account of sex determination was worked out in 1905 by Miss N. M. Stevens, using several insects as her material. Shortly afterwards E. B. Wilson traced a similar story in other insects. We may take as our example the fruit fly Drosophila reported by Stevens in 1907 and 1908.
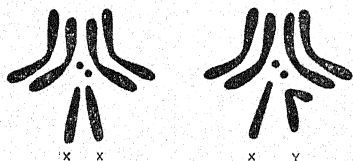
Fig. 38. The chromosomes of *Drosophila*. (From Bridges in *Genetics*.)

In Drosophila there are four pairs of chromosomes (Fig. 38). Three of these four pairs consist of exact mates in both sexes, each sex containing two pairs of V-shaped chromosomes and a small pair of dot-like chromosomes. The remaining pair does not agree in the two sexes. In the male one member of this pair is straight (the X); the other is bent and with unequal arms (the Y). In the female there are two X's instead of an X and a Y.

A special technique is necessary in searching for chromosomes. A small piece of tissue is sliced into sections so thin that the cells are seen in a single layer when they are examined under the microscope. The sections are also treated with special stains that bring out the chromosomes. The best animal tissue in which to see chromosomes as a rule is the testis. But the tissue has to be fresh; otherwise the chromosomes clump up and are difficult to study in minute detail.
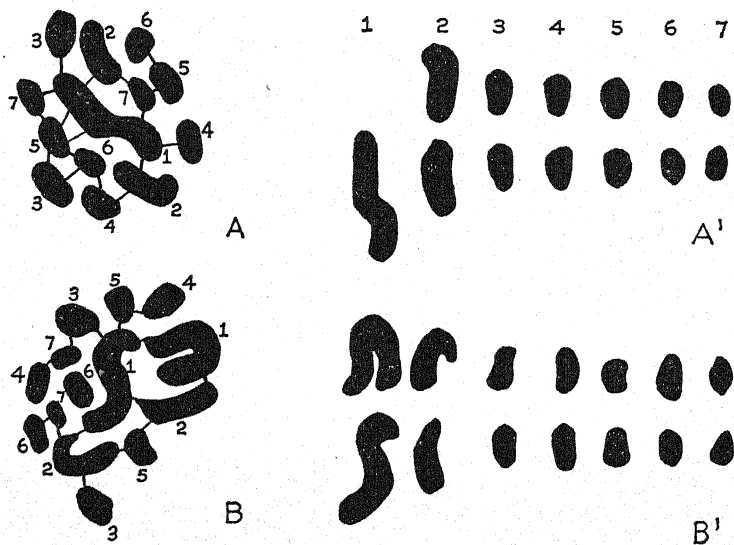
Fig. 39. The chromosomes of a bug (*Protenor*). Male, above (A, A'); female, below (B, B'). No. 1 is the *X* (unpaired in the male). (From Wilson, *The Cell*, by permission of The Macmillan Company, publishers.)

In the case of man it was necessary to get the testes from recently executed criminals or from persons who were castrated on some medical ground. It was not easy to get fresh material in the necessary amount for repeated and careful examination, and unless lower animals had already paved the way and given biologists an idea of what to look for, it would have been very difficult to discover the sex chromosomes in man.

Sex has been found to be determined by chromosomes in numerous other forms of life besides Drosophila and man, including various insects, fishes, birds, a number of mammals (including monkeys), and plants in which the sexes are separate. Figure 39 shows the sex chromosomes of a bug (*Protenor*).

Sex-linked Inheritance in Man.—Our knowledge of the X chromosome has shed light on the inheritance of color blindness in man. Women are much less often color-blind than men. But if a woman does happen to be color-blind, and if she marries a normal man, all of her sons are color-blind but none of her daughters are.
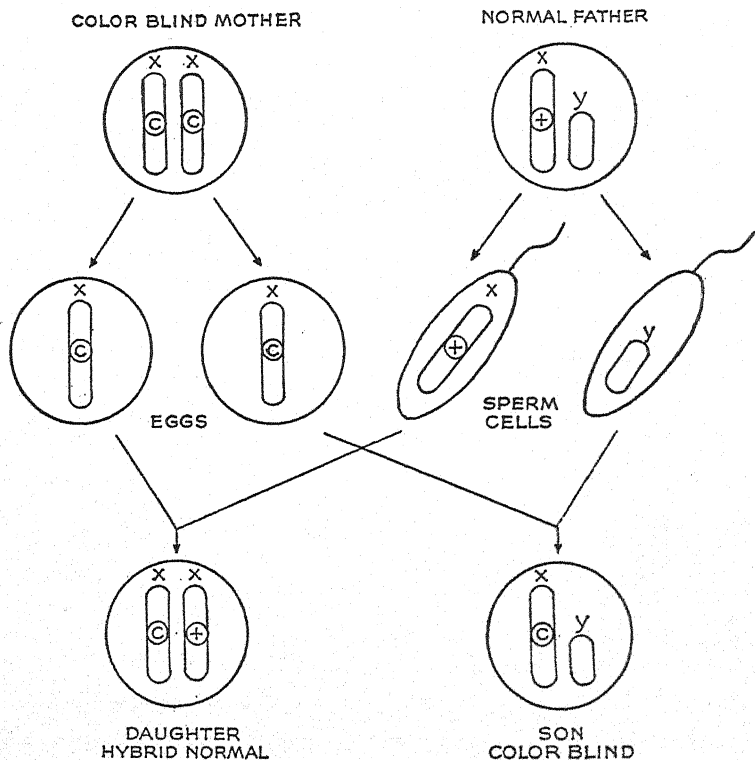
Fig. 40. The mating of a color-blind woman and a normal man.

We can readily understand this on a simple basis. The X chromosome contains genes not only for sex but also for other traits, such as normal color vision. A recessive mutation in the X caused color blindness. We can designate the mutated gene as c (for color blindness), and its normal allele as $+$. A color-blind woman carries a c in each of her X's (Fig. 40). Her formula is c/c. A normal man carries $+$, the normal allele of c, in his X. We may regard his Y chromosome as an empty sack that carries practically no genes.

Thus his formula is $+/$ (the blank space below the line indicating the "empty" Y). The sons in the mating under consideration receive their X from their mother. As this contains c, they are colorblind. The daughters on the other hand receive an X from their
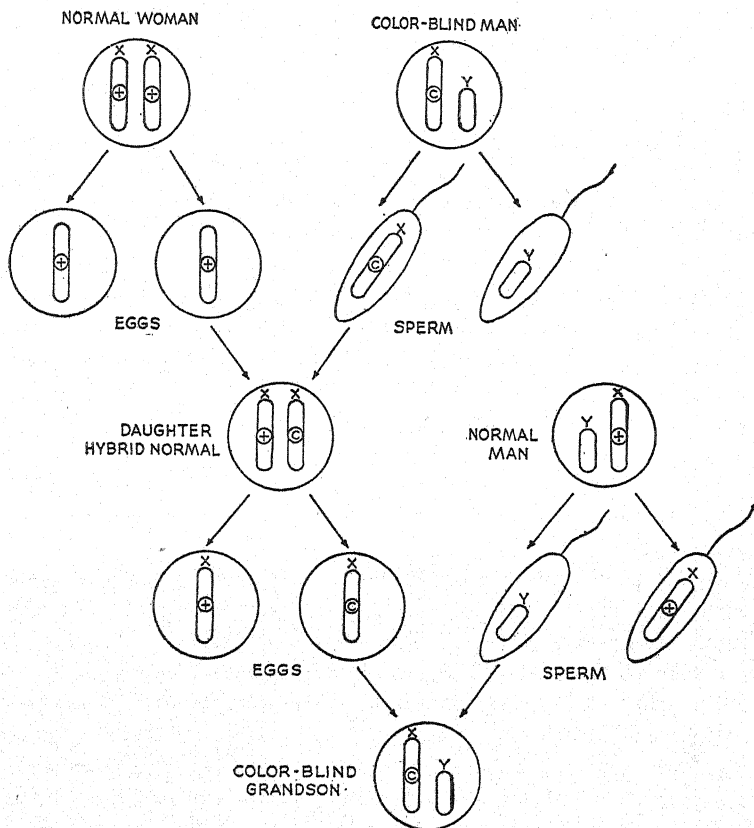
Fig. 41. The transmission of the color-blind gene from a man to half his grandsons through his daughters.

father (in addition to one that they receive from their mother), and as the paternal X contains $+$, the daughters are normal appearing.

Consider next the mating of a pure normal woman with a colorblind man (Fig. 41). The daughters from this mating are $c/+$ (hybrid normals) and the sons (not shown in Fig. 41) are $+/$ (normal). If the daughters should marry normal men ($c/+$ ♀ × $+/$ ♂), then half of *their* sons would be color blind ($c/$). The

other half (not shown in Fig. 41) would be normal $(+/ \ )$. All of the second generation daughters would be normal $(1 \ +/+ : 1 \ c/+)$, though half would be hybrids.

It is apparent now why color blindness is much scarcer among women than men. For a marriage to produce color-blind males it is necessary that only one of the parents, the mother, carry the color-blind gene. But if the marriage is to produce color-blind females both parents must carry the gene. Any such coincidence would be very unusual, unless the parents were related.

Mutations for a number of other abnormalities besides color blindness are known to occur in the sex chromosomes of man; among these are the abnormalities known as hemophilia and night blindness. Persons afflicted with hemophilia have a tendency to profuse bleeding from slight wounds because their blood fails to clot upon being shed. In night blindness unusual difficulty is experienced in seeing in subdued light. (The rods in the retina are affected in night blindness, the cones in color blindness.) Both the genes for hemophilia and night blindness are recessive to their normal alleles.

Genes which are in the X chromosome are said to be *sex-linked.* They are tied or linked to sex in inheritance and are said to show *sex linkage.*

Sex-linked Inheritance in Drosophila.—The study of lower animals again made it possible to understand such a case as color blindness in man, and it was the insect Drosophila that furnished the clearest material. Numerous mutations have occurred in the X chromosome of Drosophila, as for example one that changed the eye color from red to white. The mutant gene white (w) is recessive to its normal allele $(+)$. When a white-eyed female is crossed to a red-eyed male $(w/w \ ♀ \times +/ \ ♂)$, the daughters are $w/+$ (hybrid red) and the sons are $w/$ (white).

In all forms of life with sex chromosomes there probably are sex-linked genes. But before a sex-linked gene can be discovered it is necessary to have a sex-linked mutation and to make the appropriate crosses of the mutant form to the normal. Sex linkage has been discovered in several forms of life besides Drosophila and man, including such diverse forms of life as chickens, moths, and plants with separate sexes.

Sex-linked Lethals.—In Drosophila occasionally half the sons in a family are missing. The sex ratio in other words is 2 females : 1

male. In a small family it might of course happen as a matter of
chance sometimes that there would be only half as many sons as
daughters. But the number of offspring in a Drosophila family
might be very large (three or four hundred) and if such a family
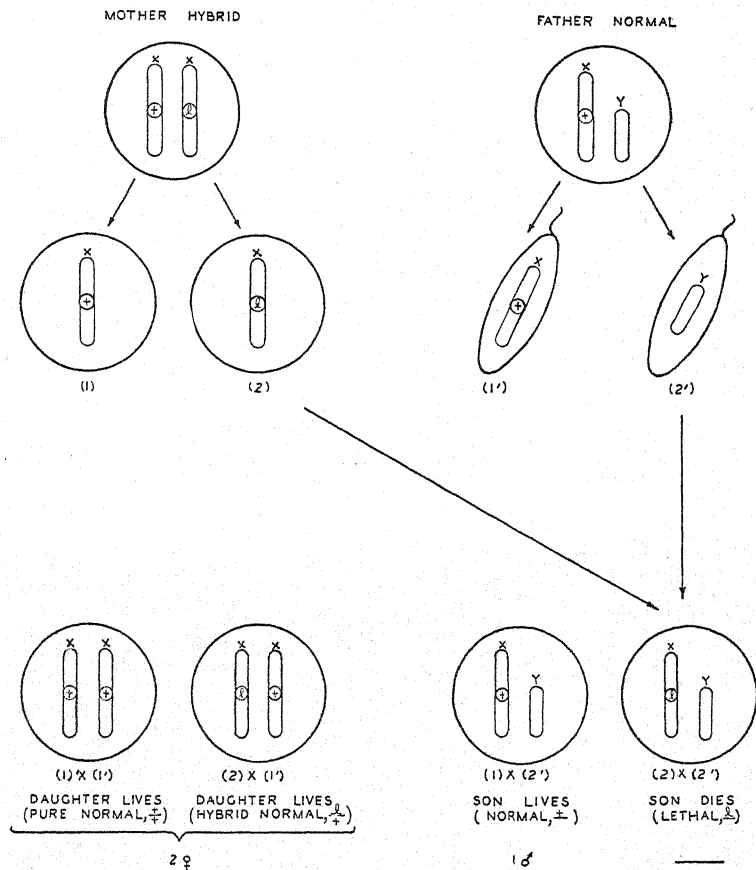
FIG. 42. A sex-linked lethal.

contained only half as many sons as daughters it would be safe to
conclude that the 2 : 1 ratio was actually significant.

In the families under discussion the mother contains a mutation
known as a *lethal* in one of her *X*'s (Fig. 42 upper left). A lethal, as
the term implies, expresses itself by causing the death of the devel-
oping young. The hybrid mother survives because she contains

the dominant normal allele $(+)$ in one of her X's, and this prevents the lethal from expressing itself. But half of her sons receive her lethal-containing X and die (Fig. 42 lower right). The remaining offspring receive at least one normal X and survive. Hence a sex ratio of 2 ♀ : 1 ♂.

The Distribution of the Sex Genes in the X Chromosome. —It is possible to break up the X chromosome into segments by means of X-rays (applied to the entire animal), and it is then found that the sex-determining material is not limited to any one segment. This can be shown by means of sexually abnormal flies known as "intersexes." An intersex, as the term implies, is intermediate between a normal male and female in sex development. It has a certain chromosomal composition, different from that of normal males and females (to be considered later). By the proper experimental procedure it is possible to get flies which contain the usual chromosomes of an intersex and in addition a fragment of the X. It is then found that the fly develops more completely into a female. Moreover, fragments from almost any part of the X, if added to the intersex formula, make for more complete female development. This therefore shows that there is no one "primary" gene for sex.

The Y Chromosome.—The Y chromosome does not have very much to do with sex development in most animals. It often is smaller than the X and in some species it is entirely missing. It is possible in fact, as Wilson showed, to arrange a number of species in a series beginning with those that have a Y chromosome as large or almost as large as the X down through intermediate steps to those that have no Y at all. In all these cases, however, the male has an X. Occasionally in Drosophila a male lacks the Y (but not the X), yet it is normal in every respect but for the fact that it is sterile because of immobility of its sperm cells. The Y contains about 8 to 10 genes necessary for the mobility of the sperm cells in Drosophila.

About 98 per cent of the Y is inert in Drosophila and is produced by about a half dozen genes each of which produces a segment or "block" of inert material about itself. The "block" genes (as they are called) have no effect on development proper but the removal of each one causes a certain reduction in the size of the Y (equal to the amount of inert material that the gene produces). There are,

however, a few active genes of the ordinary kind in the *Y*, as for instance, one that is concerned with bristle development.

The Theory of Genic Balance in Sex Determination.—Sex like everything else depends upon a large number of genes and these are distributed throughout all the chromosomes, autosomes as well as sex chromosomes. But the sex genes in the *X* are of such a nature that one dose of them in conjunction with the autosomes causes one sex to develop; two doses—again in conjunction with the autosomes—the opposite sex. This was pointed out by E. B. Wilson in 1909.

If sex development depends on the interaction of the *X* and the autosomes, then obviously it depends on the *relative* amounts of *X* and autosomes, not on the absolute number of *X*'s (whether one or two). Thus one *X* produces a male if it interacts with two sets of autosomes; but if it interacted with one set, we should ordinarily expect it to produce a female, because one *X* and one set of autosomes is relatively the same as two *X*'s and two sets of autosomes. In 1912 H. J. Muller proposed the theory (unpublished) that sex in Drosophila and other animals having the usual type of sex determination depends upon the *relative* amount of *X* and autosomes. This we may designate as the theory of genic balance. The term "genic balance" was coined by Bridges following the announcement of the theory.

In the Drosophila type of sex determination, the addition of an *X* throws development in favor of femaleness in that the autosomes plus one *X* cause maleness, but the autosomes plus two *X*'s cause femaleness. On the other hand in the male there is relatively more autosomal material as compared to the *X* than in the female, and so the addition of autosomes to a cell favors maleness. We might therefore think of the *X* chromosomes as containing the genes for femaleness and the autosomes the genes for maleness. However, it is not likely that all the female determiners are in the *X* and all the male in the autosomes. More likely determiners of both types are in both the *X* and in the autosomes, but the female determiners predominate in the *X*, the male determiners in the autosomes. In other words the *net* effect of increasing the sex genes in the *X* is to throw the balance of sex development in favor of femaleness, and the net effect of increasing those in the autosomes is to throw the balance in favor of maleness.

The Difference in the Sex Formula in Different Forms of Life.—The chromosomal difference between the sexes in lower animals is not always the same as in Drosophila and man. In birds, moths, and butterflies the female is XY, the male XX (Fig. 43).

It might seem rather odd that there should be two different formulas for sex determination in the animal kingdom. But the two have a point in common. In both a certain chromosome throws the developmental balance in favor of one sex or the other. The sex with an XX pair is said to be the *homozygous sex*, the one with a single X (or an XY pair) the *heterozygous sex*. If we simply indicate which is the heterozygous sex, as is often done, then of course the opposite sex is the one that is homozygous.

FEMALE   MALE

Fig. 43. The sex chromosomes in chickens. (From Hance in *Jour. of Morphology*.)

Closely related forms of life may differ in their sex formula. In some fishes the male is the heterozygous sex; in others, the female is. The male is the heterozygous sex among some insects (flies, bugs, beetles, and grasshoppers), the female among others (moths and butterflies). These facts would indicate that perhaps sex determination sometimes arose independently in closely related forms of life, for then it would be expected as a matter of chance that the formula would not always have been the same. This sort of thing has been found to apply to special strains of corn with separate sexes, discovered by Jones. It is also possible that sometimes the sex formula changed about. In fishes Winge has actually found that the sex formula might readily be changed about, as will be shown later.

Sex Linkage in Birds and Moths.—In chickens a dominant mutation in the X chromosome changed uniform black feathers to white feathers with black bars, as seen in barred Plymouth rocks. We can designate the mutant gene as Ba (barred) and its normal allele as $+$ (Fig. 44). The inheritance of Ba conforms to the fact that in chickens the male is XX and the female XY. Thus, when a black male is crossed to a barred female, the sons are barred and the daughters black (Fig. 44). When a pure barred male is crossed to a black female ($Ba/Ba\ \male \times +/\ \ \female$), the sons are

$Ba/+$ and the daughters $Ba/$. Thus both sons and daughters are barred.

In the currant moth (*Abraxas*) a recessive mutation in the X caused the color to change from dark to light. (The normal dark race is known as *A. grossulariata*, the light race as *A. lacticolor*).

FIG. 44. The cross of a black male chicken and a barred female.

We can designate the mutant gene as l (light) and its normal allele as $+$. Since the male in moths is XX, a light male is l/l. The cross of a light male by a dark female is $l/l \, \male \times +/ \quad \female$. The F_1 are $l/+$ (dark sons) and $l/$ (light daughters). This case is like that of color blindness in man except that the sexes are reversed with regard to which carries one X and which carries two X's.

The Derivation of the Heterozygous Sex from Breeding Experiments.—We could tell which was the heterozygous sex from breeding experiments, entirely apart from microscopic exami-

nation. Thus in chickens barred male by black female (Ba/Ba ♂ × $+/$ ♀) gives offspring all of which are barred ($Ba/+$ ♂ and $Ba/$ ♀). This cross tells us that barred is dominant, but it does not necessarily tell us that it is in the X, for if barred were in an autosome and dominant, all of the offspring would likewise be barred. But when a black male is crossed to a barred female ($+/+$ ♂ × $Ba/$ ♀), the daughters are black ($+/$). Since barred is dominant the daughters do not get the barred chromosome but only the black. Hence they have only one X. This makes them the heterozygous sex.

It so happened that the genetical results were got in advance of the cytological in the case of birds (chickens being the experimental material), and these indicated that the female was the heterozygous sex. Later some cytologists examined the chromosomes of birds and at first seemed to find that the male was the heterozygous sex (seemingly having a pair of chromosomes consisting of unequal mates). If this discrepancy between the breeding and cytological results had really held, the whole chromosome theory of inheritance would have had to be discarded. More careful examination of the chromosomes of birds under the microscope showed, however, that the female, not the male, was the heterozygous sex. Thus the genetical and the cytological findings agreed.

Sex Determination in the Hymenoptera.—A peculiar method of sex determination has been found in the Hymenoptera (bees, ants, wasps). The method in question has been found by P. W. Whiting and Anna R. Whiting in Habrobracon, a wasp that is parasitic on mealworms. The eggs of Habrobracon may develop either with or without fertilization. The unfertilized eggs develop into males. The fertilized eggs as a rule develop into females, but if the parents are closely related then some of the fertilized eggs develop into males. Accordingly, the males are either haploid or diploid, depending on whether they develop from unfertilized eggs (with one set of chromosomes) or from fertilized eggs (with two sets). The females, however, are always diploid.

Whiting has found that in Habrobracon there is a certain locus to which a number of different genes belong (a, b, c, d, etc.). These genes constitute a series of alleles, since by definition alleles are genes that belong to the same locus. A given individual might contain any one member of the series in question, or, if heterozygous, any two. Whiting has found that females are always heterozygous

at the locus in question (as a/b). Diploid males, on the other hand, are homozygous (as a/a or b/b). Haploid males are in a sense the equivalent of homozygous diploids, since both have only one kind of allele (a or b). The locus under discussion can be referred to as the *sex locus*.

Inbreeding would often produce homozygous diploids. Thus suppose that a queen is a/b. If she received a from her mother, then some of her brothers would also have a (since they have the same mother), and the mating $a/b \times a$ would produce some fertilized eggs homozygous for a (a/a). These would develop into males. If, on the other hand, a queen of composition a/b were mated with an unrelated male, say, one with c in the sex locus, then all the fertilized eggs would be heterozygous (a/c or b/c), and they would all develop into females. Thus in Habrobracon sex is determined by homozygosity or heterozygosity at a specific sex locus.

Whiting has suggested that the "locus" in question is really a small segment of a chromosome (the "X segment") and that the so-called alleles (a, b, c, etc.) are at different loci within the X segment. If we confine ourselves to just two loci (a and b), then an X segment with a would be normal at the b locus ($a +$); and one with b would be normal at the a locus ($+ b$). The combination of the two segments would be $\dfrac{a +}{+ b}$. The $+$ alleles are dominant and it takes both to produce a female. Hence $\dfrac{a +}{+ b}$ is a female. But either mutant (a or b) produces a male. Thus $a +$ is a male (haploid); so is $\dfrac{a +}{a +}$ (diploid). This situation is somewhat comparable to the production of white flowers in sweet peas by either of two recessive mutations. Purple would correspond to the female and either white race to the male.

It has long been known that in bees the fertilized eggs develop into females (either workers or queens, depending on the feeding) and the unfertilized eggs into males (the drones). Possibly sex is determined in bees in essentially the same way as in Habrobracon. But diploid males have not been reported in bees. Perhaps bees seldom inbreed. If so, we could account for the absence of diploid males among them.

Sex Determination in Plants.—In some flowering plants the stamens and pistils are in separate flowers and in separate plants,

and then we may speak of the plants as male and female. When the sexes are separate they often show a chromosomal difference. Usually the male is XY, the female XX.
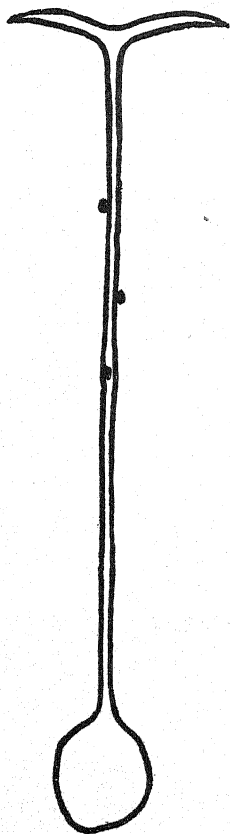

In Melandrium (a flowering plant) Warmke has found that the Y takes an active part in sex determination. Thus for example Warmke was able to get plants with 2 X's and a Y. These develop into males, although they would have developed into females if the Y had not been present. It is also possible to get plants that have two X's and four sets of autosomes (instead of the normal two). These develop into females, although they have only one X to two sets of autosomes, the same proportion as in a male. Thus sex is not determined by the proportions of X and autosomes, as in Drosophila, but by the interaction of X and Y.

Sex Determination in Bonellia.—External conditions rather than the chromosomes may in exceptional instances determine sex. There is a curious case of this sort in a worm known as *Bonellia* (Fig. 45). In Bonellia the males are very small by comparison with the females. They live in close association with the females, at first on the proboscis of the female and later in her oviduct. When the animal is very young (a larva) it is neither male nor female, and it may do one of two things. It may find its way to the proboscis and later to the uterus of a fully developed female, in which case it becomes a male; or it may be independent and grow to a full-sized individual, a female. The environment of the growing larva is different in the two cases. In the first the female constitutes part of the larva's environment; in the second, she does not. The larva accordingly develops into a male or into a female as the case may be. Here then the environment determines sex. But this method of

FIG. 45. *Bonellia.* A female and three males (shown as specks on the proboscis of the female).

sex determination is very exceptional. Usually sex is determined by chromosomes.

Summary of Mechanisms of Sex Determination.—In summary, sex may be determined by (1) genic balance between X and autosomes (Drosophila), (2) homozygosity or heterozygosity at a sex locus (Habrobrachon), (3) the interaction of the X and Y (Melandrium), (4) the environment (Bonellia). Of these various mechanisms the one found in Drosophila is the usual one for animals; that is, the mechanism of genic balance between X's and autosomes.

The Advantage of Chromosomes as a Method of Sex Determination.—Perhaps early in the course of evolution sex was not determined by chromosomes but by external conditions. Certain conditions, such as plenty of food and low temperature, might have favored the production of large sluggish cells; and the opposite conditions small, motile cells. These would accordingly have been female or male reproductive cells, respectively, and the animal or plant that produced them, female or male. But external conditions are rather unreliable as a means of producing the opposite sexes in approximately equal numbers. A long cold spell for instance would cause an excess of one sex, if cold favored the production of that sex. Chromosomes on the other hand could bring about a 1 : 1 sex ratio with certainty and so chromosomes would come to provide the mechanism of sex determination through the natural selection of mutations that led to a chromosome mechanism. One of the sexes came to contain an XX pair of chromosomes, the other an XY pair.

Undoubtedly the Y was at first very much like the X (as it often is today in fishes), but in the course of time it got smaller than the X in many species and even disappeared entirely, or it came to contain a good deal of inert material, as in Drosophila, so that in effect it became virtually an empty sack.

Triploid Intersexes in Drosophila.—The theory of genic balance was unexpectedly confirmed by Bridges with findings made on Drosophila some nine years after the theory was first announced. In Drosophila sometimes a female accidentally has three chromosomes of each kind instead of the normal two. This is referred to as a *triploid* female. A triploid is usually produced by the union of a normal X-containing sperm cell and an egg which

through some accident in cell division came to have the double chromosome number.

When the reduction division takes place in a triploid female one X goes to one pole and two to the other. Half the eggs therefore receive one X and the other half two. The same sort of thing applies to the distribution of any of the other chromosomes. As a result a triploid female forms various classes of eggs having all possible combinations of one or two of a given kind of chromosome with one or two of every other kind. Some happen to get one X but two 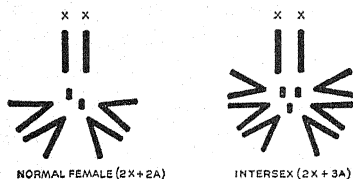autosomes of each kind. We could designate these as $1 X + 2 A$ (where $2 A$ stands for two sets of autosomes). When a $1 X + 2 A$ egg is fertilized by a normal X-containing sperm cell (or $1 X + 1 A$ sperm) it gives rise to offspring of composition $2 X + 3 A$ (Fig. 46). Here the relative proportions of X and A are intermediate between that of a normal male $(1 X + 2 A)$

NORMAL FEMALE (2X+2A)   INTERSEX (2X+3A)

FIG. 46. Chromosome diagrams of a normal female *Drosophila*, and of an intersex.

and a normal female $(2 X + 2 A)$. These relative proportions could be expressed as $3 X + 6 A$ (or $1 X + 2 A$), $4 X + 6 A$ (or $2 X + 3 A$) and $6 X + 6 A$ (or $2 X + 2 A$); and $4 X$ is in between $3 X$ and $6 X$. Accordingly a $2 X + 3 A$ fly is sexually intermediate between a normal male and a normal female and is known as an *intersex*. In particular, its reproductive gland is intermediate between a testis and an ovary and it has a mixture of other male and female sexual characters. On the whole, organs that begin their development later are more likely to be of male type in an intersex. It appears as though the egg starts its development as a female and then changes over to a male, parts laid down after a certain stage being of the male type. The stage at which the change-over takes place is called the *turning point*. The time in development when the turning point occurs varies from one fly to another according to modifying genetic and environmental factors (including the content of the original egg cytoplasm, conditioned by the genes of the previous generation).

In addition to intersexes, triploid females produce other unbalanced classes of offspring, two of which are of interest in the present connection. One of these is $3 X + 2 A$ (produced by the union of a

$2 X + 1 A$ egg and a $1 X + 1 A$ sperm cell). Another unbalanced class is $1 X + 3 A$ (from a $1 X + 2 A$ egg fertilized by a Y-containing sperm, or $0 X + 1 A$). In the first of these ($3 X + 2 A$) the relative amount of X material is greater than that in a normal female. As a female normally has relatively more X material than the male, a $3 X + 2 A$ egg would be a "super-female" and is designated as such. But she is fairly normal in appearance and in development of sex organs, though usually sterile and of low viability, and is really a super-female only from the standpoint of chromosomal make-up. In a fly of class $1 X + 3 A$ the relative amount of X is less than that of a normal male. As the male normally is characterized by having comparatively less X material than the female, $1 X + 3 A$ is a "super-male." But this class appears much like the normal male in sexual characteristics, though sterile (unlike what the term super-male should connote) and of very low viability.

The triploid female itself ($3 X + 3 A$) has the normal proportions of X and A and accordingly is a normal female in outward appearance. Cells having just $1 X$ and $1 A$ also have the normal proportions of X and A, and occasionally a patch of cells in a female contains just one X and one set of autosomes; that is, the patch is haploid (being produced apparently from a cell which underwent an irregular division in the developing fly). Such haploid patches have smaller cells and they have smaller ommatidia, bristles and other parts whose size depends on that of the individual cells; but otherwise the patches in question are of normal female appearance, as for instance in regard to lack of "sex combs," which are normally lacking in the female. In one case, observed by Lamy and Crew, approximately half of a fly was haploid (with one X and one set of autosomes). It was female tissue and produced a few fertile eggs. All this again is in agreement with expectation on the theory of genic balance.

The offspring of a triploid female in Drosophila give strong confirmation of the theory of genic balance. For we see that a normal female is not simply produced by $2 X$'s but by the X's and autosomes in the proper proportion to each other. The triploid and haploid females show that the absolute number of X's may be changed and yet a normal female may be produced, provided the relative proportion of X to autosomes is not changed.

Intersexes in the Gypsy Moth (Lymantria).—Intersexes occur in the gypsy moth *Lymantria dispar*, and in this species they

have been studied in great detail by Richard Goldschmidt. The normal female and male gypsy moths differ very distinctly (Fig. 47.1 and .2, upper middle moths). By crossing gypsy moths from different parts of the world it is possible to produce intersexes, as
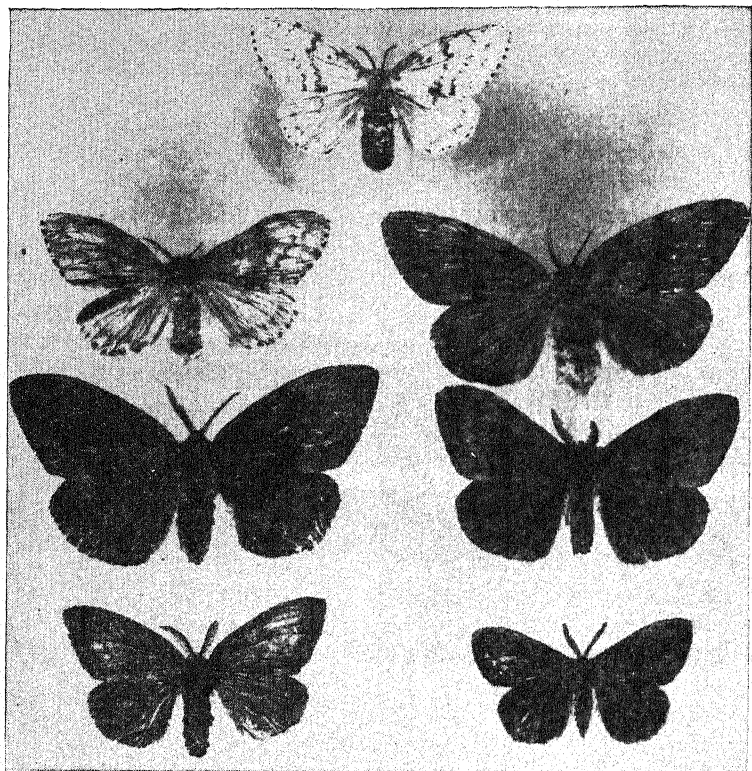
FIG. 47.1. Female intersexes in the gypsy moth. A normal female is shown above. (From Goldschmidt.)

for example by crossing a European female by Japanese male. In Fig. 47.1 the moths shown directly below the normal female were produced by such crosses. They started their development as females but later developed male traits, those further along in the series having developed more and more male traits. The lower right moth of this series changed over completely to a male and is called a male by "sex reversal." The originally female moths with various amounts of male development, but not completely male, are called

"female intersexes." In some crosses the males develop female traits and are called "male intersexes." In Fig. 47.2 the moths
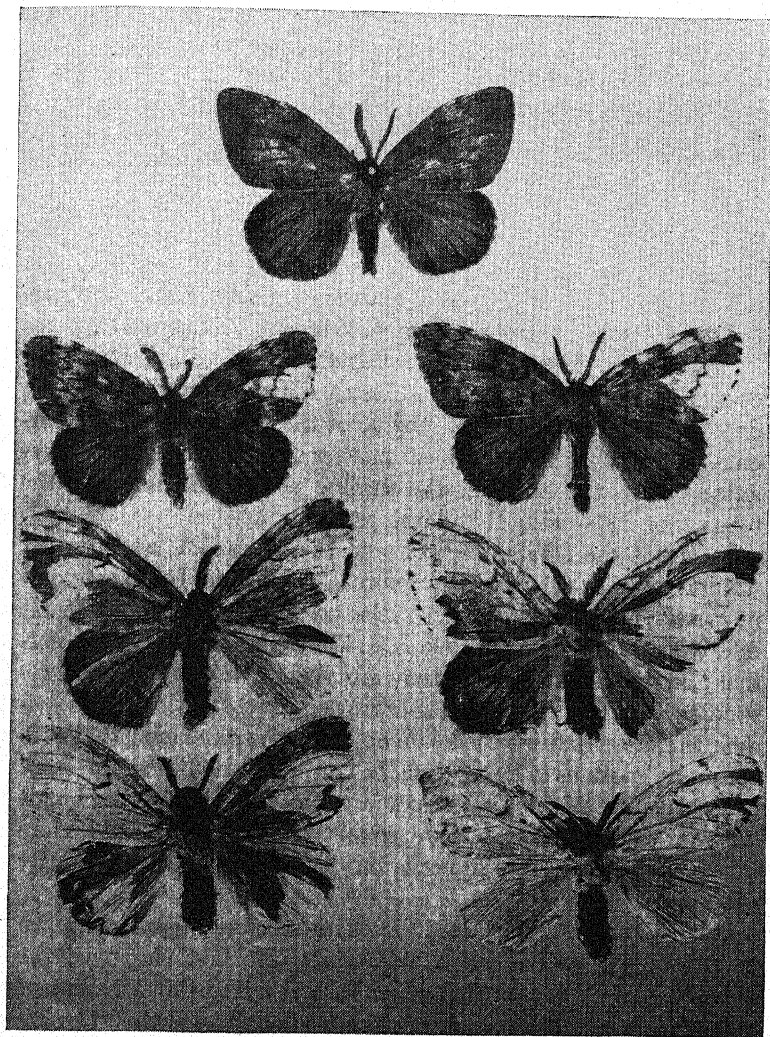

FIG. 47.2. Male intersexes in the gypsy moth. A normal male is shown above. (From Goldschmidt.)

shown directly below the normal male were originally male but later developed female traits. They constitute a series of male intersexes with increasing amounts of female development.

In the gypsy moth there are two opposing sets of sex factors: those for maleness (M) and those for femaleness (F). These differ in strength from one race to another. They are weak in the European races, strong in the Japanese. Normally, the weak female determiners go with the weak male, and strong female with strong male. But when two races differing in strength are crossed, it is possible to get new combinations which produce intersexes. Thus, when a European female is crossed to a Japanese male, the daughters receive weak female tendencies from their mother but strong male from their father, and this combination produces female intersexes.

Goldschmidt has shown that the female tendencies at first predominate in a female intersex, but they are slow-working and are overtaken by the faster-working male tendencies derived from the Japanese race. Male characters therefore start to appear at the "turning point." This point varies in different intersex females, depending on the relative strengths of the sex factors in the parents. The weaker the European mother and the stronger the Japanese father, the earlier is the turning point and the larger the amount of male tissue that develops.

In moths the females are XY and the males XX, and this sex formula applies to the gypsy moth in particular. Intersex gypsy moths have the same formula as the normal gypsy moths, female intersexes being XY and male XX. Moreover, they have the normal number of autosomes—they are diploid throughout. In Drosophila, we just saw, the intersexes were partly triploid. It is evident therefore that intersexes do not have the same explanation in the gypsy moth as in Drosophila. In fact, we do not know the exact genetic constitution of intersexes in the gypsy moth. Goldschmidt assumes that femaleness is dependent on determiners which are inherited through the cytoplasm, but his crosses do not necessarily prove this.

Intersexes were discovered in *Lymantria* before they were known in Drosophila, and it was Goldschmidt who first employed the term "intersex" for an individual with a mixed development of male and female traits. Goldschmidt also discovered the "turning point," and he accounted for it in terms of the relative rates at which opposing male and female tendencies express themselves in development.

Intersex Males in Pigs.—Suppose a mutation increased the effectiveness of the sex genes in the X. It would thereby make one

X approach two normal X's in strength, and so it would make an animal which carried one X intermediate between a male and a female, in a species in which normally $1\,X = \sigma$, $2\,X = \circ$. Or, suppose that a mutation weakened the effect of the sex genes in the autosomes. It would thereby make the X relatively stronger and so again it might make an intersex out of an animal with one X.

It is in fact known that a single mutation might cause intersexes. In the New Hebrides (a group of South Sea Islands) there is· a stock of pigs which produce intersex males. The sex formulas for pigs (and for mammals in general) is male $= XY$, female $= XX$. An intersex pig is XY. It contains a sex linked recessive mutation (i) which causes it to be an intersex instead of a normal male (possibly by strengthening the X or weakening the autosomes). An intersex is sterile, but a hybrid female ($i/+$) is fertile (since i is recessive), and when the hybrid is mated to a normal male ($i/+ \circ \times +/ \sigma$) half the male offspring are intersex males ($i/$). Half the female offspring are hybrids ($i/+$), and they in turn produce intersex males and hybrid females when mated to normal males. Thus they continue the stock.

The intersexes produced by the cross of gypsy moths are not due to a single mutation, for each race when bred to itself is normal and hence does not carry a gene for the intersex condition.

Sex Reversal in Fishes.—A case of sex reversal has been reported in fishes by O. Winge. In *Lebistes* (a minnow-like fish) the formula for the sexes normally is female $= XX$, male $= XY$, but in Winge's cultures some XX males appeared. When bred to normal females (XX) they produced only XX offspring, all of which were females. The stock was reproduced by breeding the original XX males to their daughters and granddaughters. At first all of the offspring continued to be XX females but later an XX male appeared among them, and this when bred to the XX females produced male and female offspring in approximately equal numbers.

In explanation of this case Winge supposes that all the chromosomes, including the Y, contain sex genes. At some loci a fish may be heterozygous for both male and female genes. Moreover, the sex genes at different loci differ in sex potency. Thus, for example, at one locus a male gene, or a female, might have a potency of "4"; at another, "20." Male and female genes have opposite effects on sex development, and Winge indicates them by opposite signs ($+$ for

male, — for female). If a fish had an excess of male genes (+) it would develop into a male; if an excess of female (−), into a female. Winge gives the following numerical examples (hypothetical) in illustration of his theory (sex chromosomes being indicated by X and Y, autosomes with female genes by large letters, those with male genes by small letters):

(1) Normal XX ♀.

X X	A a	B B	c c	D D	E e	F f
− −	− +	− −	+ +	− −	− +	− +
12 12	20 3	6 6	4 4	2 2	3 2	1 1

Sum: $+14 - 64 = -50$ (a female).

(2) Normal XY ♂.

X Y	A a	B b	c c	D d	e e	F F
− +	− +	− +	+ +	− +	+ +	− −
12 70	20 3	6 3	4 4	2 3	2 2	1 1

Sum: $+91 - 42 = +49$ (a male).

Ordinarily the female sex genes predominate in the X (each X in the above example being -12). The female genes also ordinarily predominate in the autosomes (the autosomes by themselves containing an excess of -26 in the first example above, an excess of -9 in the second example). The male genes predominate in the Y (being $+70$ in the second example above). The autosomes in combination with XX make a female and in combination XY a male, the Y throwing the balance in favor of the male. But at some of the autosomal loci a fish is heterozygous for both male and female genes, and by crossing and Mendelian recombination an unusually large proportion of male genes might accumulate in the autosomes, making the fish a male, even though it has two X's. Thus (4) below is a normal female, (5) a normal male; but by crossing (4) and (5) we might (by Mendelian recombination) get (6).

(4) A possible normal female (XX).

X X	A a	B b	c c	D d	e e	f f
− −	− +	− +	+ +	− +	+ +	+ +
12 12	20 3	6 3	4 4	2 3	2 2	1 1

Sum: $+23 - 52 = -29$ (a female).

(5) A possible normal male (XY).

$X\ Y$	$A\ a$	$B\ b$	$c\ c$	$d\ d$	$E\ e$	$f\ f$
$-\ +$	$-\ +$	$-\ +$	$+\ +$	$+\ +$	$-\ +$	$+\ +$
12 70	20 3	6 3	4 4	3 3	3 2	1 1

Sum: $+94 - 41 = +53$ (a male).

(6) An XX male representing a possible recombination class got by crossing (4) and (5).

$X\ X$	$a\ a$	$b\ b$	$c\ c$	$d\ d$	$e\ e$	$f\ f$
$-\ -$	$+\ +$	$+\ +$	$+\ +$	$+\ +$	$+\ +$	$+\ +$
12 12	3 3	3 3	4 4	3 3	2 2	1 1

Sum: $+32 - 24 = +8$ (an XX male!).

By means of crossing we might substitute for one of the a's in formula (6) above an A of value -20, giving

(7)

$X\ X$	$A\ a$	$b\ b$	$c\ c$	$d\ d$	$e\ e$	$f\ f$
$-\ -$	$-\ +$	$+\ +$	$+\ +$	$+\ +$	$+\ +$	$+\ +$
12 12	20 3	3 3	4 4	3 3	2 2	1 1

Sum: $+29 - 44 = -15$ (a female).

Crossing an XX male of formula (6) with an XX female of formula (7) would give 50 per cent female and 50 per cent male offspring (as can easily be verified by the reader). Thus a pair of autosomes has now become a pair of sex chromosomes ($A =$ the new X, $a =$ the new Y), and the old X chromosomes have become a pair of autosomes (since there is a pair of them in both sexes, just as applies to the autosomes in general). But the female (7) is now the heterozygous sex; the male (6) the homozygous. Originally the reverse was true, since the female is XX in formula (1), the male XY in formula (2).

Thus two things have happened: (1) the sex chromosomes have changed into autosomes and, vice versa, a pair of autosomes into sex chromosomes; and (2) the female has changed from the homozygous sex (XX) to the heterozygous sex (XY).

The Conversion of an Hermaphrodite Plant (Corn) into One with Separate Sexes.—Mutations may cause an hermaphrodite to change to one sex or the other. This has been observed by Jones in corn. Normally the sexes are combined in corn. The

stamens are the male organs and are at the top of the plant, where they form the tassels. Their enlarged ends, the anthers, contain the pollen. The pistils or silks are the female organs and are clustered on the side of the plant in the position of the future ears of corn.

FIG. 48. Corn plants of opposite sex. Left, a female (tassel-seed); right, a male (barren). The normal plant is similar to the one on the right, but has silks and produces cobs with seeds. (From D. F. Jones.)

They contain the ovules at their base and from these the seeds develop. Each pistil contains one ovule and gives rise to one seed, the whole cluster of pistils giving rise to the seeds on a single ear of corn.

A recessive mutation caused the loss of silks (pistils) and so made the plant barren (Fig. 48). We can designate the mutated gene as barren (*ba*). A recessive mutation in another chromosome caused pistils (and seeds) to develop on the tassels in place of the pollen-containing organs (the anthers), and the mutated gene is designated as tassel-seed (*ts*). The tassel-seed plant still has its normal silks (on the side of the plant) and the mutation does not prevent the normal development of these silks into seeds. Therefore a plant pure for *ts* develops seeds both on its cobs and on its tassels. If we designate the normal alleles of *ba* and *ts* by $+$ signs, then a pure normal plant is $\dfrac{+\ +}{+\ +}$, a barren (silkless) plant is $\dfrac{ba\ +}{ba\ +}$, and a tassel-seed plant is $\dfrac{+\ ts}{+\ ts}$. A barren plant $\left(\dfrac{ba\ +}{ba\ +}\right)$ is a male because it has no silks or female organs, but it still has tassels—the male organs. A tassel-seed plant $\left(\dfrac{+\ ts}{+\ ts}\right)$ is a female because it lacks male organs. A plant can be got which is pure for both mutant genes $\left(\dfrac{ba\ ts}{ba\ ts}\right)$. This plant produces corn on its cobs and tassels despite

the fact that it is pure for ba, because ba (barren) is without effect in a plant pure for ts (tassel-seed). We can think of ts as suppressing ba; or perhaps as making seeds all around, on cobs as well as tassels. In any event a plant of composition $\dfrac{ba}{ba}\dfrac{ts}{ts}$ produces only seeds. Hence it is still a *female*, and this is the important thing for our present purposes. A barren or male plant is pure for ba, but might be heterozygous for ts, thus $\dfrac{ba}{ba}\dfrac{ts}{+}$.

The cross $\dfrac{ba}{ba}\dfrac{ts}{ts}$ (female) $\times$ $\dfrac{ba}{ba}\dfrac{ts}{+}$ (male) yields offspring in the ratio of $1\ \dfrac{ba}{ba}\dfrac{ts}{ts}$ (female) : $1\ \dfrac{ba}{ba}\dfrac{ts}{+}$ (male). The offspring are of the same composition as their parents, and they in turn produce males and females in equal numbers. Thus a strain of corn with separate sexes has arisen from the normal hermaphroditic strain.

Tassel-seed may also be caused by a dominant mutation (Ts) in a separate chromosome from the one with ts (recessive tassel-seed), and a race of corn with separate sexes has been derived from dominant tassel-seed in conjunction with barren $\left(\varphi = \dfrac{ba}{ba}\dfrac{Ts}{+} , \right.$ $\left. \male = \dfrac{ba}{ba}\dfrac{+}{+} \right).$

Gynanders in Drosophila.—Sometimes one region of a fly is male and the rest female; for example, the left half of the body might be male and the right half female. Such flies are known as gynanders or gynandermorphs. In a gynander the line of division between male and female tissue need not necessarily be through the middle of the body. Sometimes just a quarter of the body is male and the rest female, or just a small island of tissue might be male.

A gynander begins its development with two X's. But in the course of cell division an X gets lost from one of the products of cell division (Fig. 49a). In case this should happen during the first cell division, then half of the animal would develop from the cell with 2 X's and this half would become female; the other half (with one X) would become male. The irregular cell division might occur later, rather than at the first cell division. In this event the cell that got just one X would give rise to less than half the body. It might

give rise to only a small patch of cells; and then only this small patch would be male tissue.

Suppose now that a gynander developed from a fertilized egg which had the recessive gene yellow body (y) in one X and the normal allele ($+$) in the other X. Suppose further that the X that got lost happened to be the one that contained the normal allele. Then the female tissue would be gray ($y/+$) and the male tissue
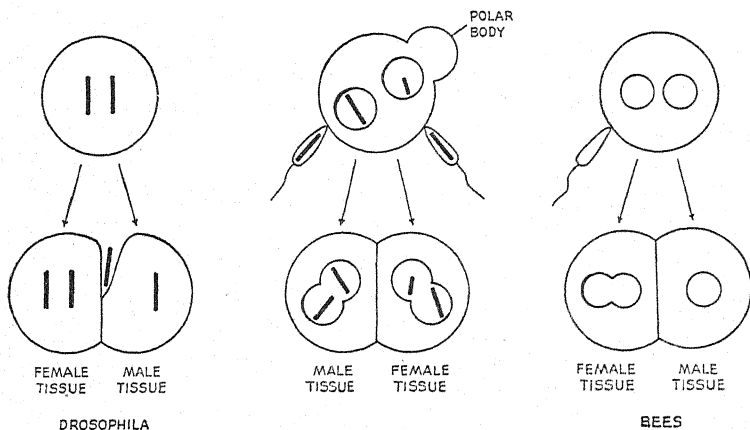

FIG. 49. Gynanders.

yellow ($y/$). In this way we could get genetic evidence that the male tissue in a Drosophila gynander was due to the loss of an X in mitosis.

Gynanders in Bees and Silkworms (from Binucleate Eggs). —Gynanders sometimes occur in bees. We do not have complete proof as to how bee gynanders are produced, but the explanation usually given is as follows. Sometimes an egg is produced with two nuclei instead of one (possibly because the polar body was not extruded or because the egg started to develop parthenogenically). If this egg were now fertilized by a single sperm cell, then only one of the two nuclei would be fertilized and this one would give rise to female tissue (Fig. 49c). The unfertilized nucleus would give rise to male tissue.

If sex is determined in bees in the same way as in Habrobracon, then another explanation of bee gynanders, suggested by Muller (unpublished), is possible. It will be recalled that in Habrobracon

females are heterozygous for a pair of alleles at a sex locus, and males are either haploid or homozygous diploids. If an egg failed to extrude its polar body in Habrobracon, it would contain two nuclei with unlike alleles (say, a and b), and if such a binucleate egg were fertilized by two sperms, each, say, with allele b, the two fertilized nuclei would then be a/b and b/b. The first would develop into female tissue (since it is heterozygous), and the second into male tissue (since it is homozygous). Possibly gynanders are produced in this manner in bees rather than in the manner first described; or perhaps they are produced in either way. In any event, they would arise from a binucleate egg. There is a race of bees, characterized by the production of a very high per cent of gynanders, due probably to a mutation which causes an unusually high production of binucleate eggs.

Gynanders are also found in silkworms. A normal female in silkworms is XY. At the reduction division the X and Y ordinarily separate, leaving the egg with one of these chromosomes (either X or Y) and a polar body with the other (Y or X). But sometimes a polar body nucleus remains in the egg, as well as the egg nucleus itself, so that the egg now has two nuclei, one with an X, the other with a Y (Fig. 49b). When fertilization takes place two sperm cells might enter the egg, each one fertilizing an egg nucleus. Since the formula for the male in silkworms is XX, each sperm nucleus would contain an X and would add its X to the egg nucleus with which it combined. One fertilized nucleus would therefore be XX and from this male tissue would develop; the other would be XY and from this female tissue would develop. A certain strain of silkworms has a tendency to form a high proportion of binucleate eggs and gynanders, due to a mutation.

The Influence of Hormones on Sex in the Vertebrates.— The ovaries and testes are sometimes referred to as the *primary* sexual characters. All other characters by which the sexes differ are then the *secondary* sexual characters. In man these include the ducts which convey the reproductive products to the outside; also the external genitalia, the voice and beard, the breasts and hips. In vertebrates in general removal of the ovaries and testes early in life interferes with the development of the secondary sexual characters. The reason for this is that the ovaries and testes produce chemical substances or *hormones* upon which the secondary sexual traits are dependent in development. These hormones are thrown into the

blood circulation by the ovaries or testes and are carried to the various parts of the body which they influence in the course of development. The hormone se-
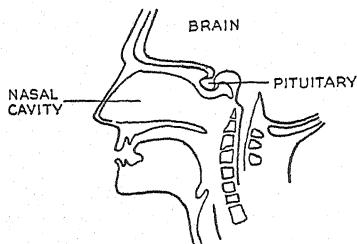

FIG. 50. Diagram showing the location and approximate size of the pituitary body.

creted by the ovaries can be artificially extracted from the ovaries (of pigs, cattle, and other animals) and is often referred to as *oestrone* (though there are a good many variants of the word, as well as of the substance). The hormone secreted by the testes is known as *androsterone* (similarly subject to vari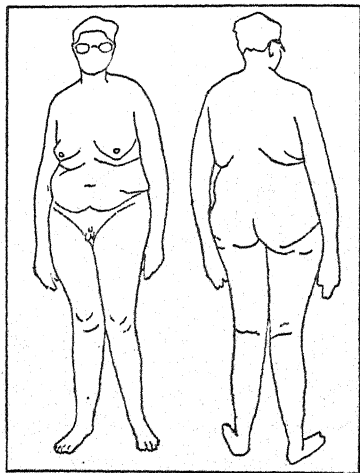ation). In rats the grafting of ovaries into a young male (after removal of the testes) causes the male to develop many female traits, at least partly. He may, for example, develop in part the genitalia of a female and small mammary glands capable of secreting some milk, and he may even have female instincts. Grafting of a young testis into an old rat is said to rejuvenate him, but the beneficial effect of "gland treatment" in man is very doubtful.

The development of the ovaries and testes themselves is dependent in part upon a small gland located at the base of the brain and known as the *pituitary* (Fig. 50). The pituitary influences the reproductive organs by means of one or more hormones which reach these organs through the circulation. If the pituitary is deranged and does not secrete its hormone, the ovaries or testes will not develop

FIG. 51. The effects of pituitary deficiency on the sexual development of a male (Froelich's syndrome). (From Wolf, *Endocrinology in Modern Practice*, by permission of W. B. Saunders and Company.)

properly. Moreover, the secondary sexual characters also will not develop properly, because they in turn depend on the secretions

of the ovaries and testes. In a human male with a pituitary deranged early in life the hips are abnormally broad, the shoulders narrow, and the breasts prominent (Fig. 51). The reproductive organs are underdeveloped. This group of abnormalities is known as *Froelich's syndrome.* A female would develop masculine traits. Derangement of the pituitary is not always the cause of underdeveloped reproductive organs. Often the cause is not known.

When males or females are improperly developed as seen in Froelich's syndrome, they are not ordinarily regarded as intersexes because their reproductive organs, though underdeveloped, are either male or female. Moreover, their instincts are either male or female and conform with their genitals.

Intersexes have, however, been reported in man. These are usually males with many female characters. They have testes but in external appearance they are females. The testes have not descended into the scrotal sack in the normal manner but are still contained in the body proper as in the embryo. Sometimes even a uterus is partially present. The external genitalia approach the female type. The sex instincts are not definitely either male or female. According to J. S. Huxley male intersexes have sometimes been reared and married as girls. We do not definitely know what causes human intersexes, but perhaps glands located close to the kidneys known as the *adrenals* are involved. The adrenals consist of two parts having totally different functions—an outer part or cortex and an inner part or medulla. It is the outer part with which we are concerned—the adrenal cortex. The bearded women of circuses suffer from a tumor of the adrenal cortex. Perhaps human intersexes are sometimes the result of a similar tumor, but one which develops in prenatal life.

The Free-martin.—In cattle when twinning occurs one of the twins occasionally is abnormal and is known as a free-martin. This is a modified female, but its ovaries are so poorly developed that it is often difficult to recognize them as ovaries. The ducts leading from the ovaries are also poorly developed and they may even be inclined towards the male type. The same is true of the external genitalia; they too tend to be of the male type. The other twin is always a male, and it is the male that has caused the female to develop abnormally while the two were still within the uterus of the mother. It has been suggested that the male produces its effect

on the female twin through the hormone secreted by its testes (androsterone).

A male twin in cattle does not always cause a female twin to develop abnormally. It does so only when a connection is established between the circulation of the two embryos through the fusion of the fetal membranes (the chorions) that surround each embryo. Ordinarily the blood passes by way of the umbilical cord from the embryo to the membranes and then back again to the embryo. But when the two membranes are joined, the blood can circulate from one embryo to the other. The male hormone can then get into the female. This hormone favors the development of the male reproductive ducts and external genitalia and acts as a check on those of the female.

When twins are of opposite sex in cattle, the male is not abnormal. The reason is that the ovaries start to produce their secretion after the male has passed the stage when his development would be susceptible to the female hormone.

Sex Hormones in Birds.—In chickens the spurs and combs of the male are dependent upon hormones coming from the testes, as shown by the fact that spurs and combs fail to develop in a castrated male. But the plumage color characteristic of the male is not dependent on testicular hormones, for a castrated male develops male feathers. On the other hand, female feathers depend on hormones coming from the ovaries, as shown by the fact that a castrated female develops male feathers. Apparently in an uncastrated female the ovarian hormone suppresses the development of male feathers; but when the ovaries are removed, there is no ovarian hormone and male feathering is not suppressed.

In certain breeds of chickens (for example, Seabrights and Campines) the males (as well as the females) are hen feathered. This is due to the fact that the feather-producing tissues are susceptible to both the male and female hormones, as shown by Morgan in experiments involving castration. When a hen-feathered male is castrated, he develops male feathering because he now lacks the male hormones to which his feather-producing tissues (the skin follicles) are susceptible. The castrated male again develops hen feathering if the testes of another animal are grafted into him, and this is true regardless of whether the testes come from a hen-feathered male or from a male of a normal race (one in which males are male-feathered). Thus it is known that the testes of a hen-feathered male do

not produce a special kind of hormone that causes hen feathering (since a hen-feathered male with a grafted normal testis develops hen feathering), but that the tissues are susceptible to the normal male hormone. The same thing has been shown by Danforth and others by means of skin grafts. When a piece of skin from a hen-feathered male is grafted on a normal male, it continues to develop hen feathering; but it develops male feathering if the normal male is castrated.

The tissues of the males in the hen-feathered races became susceptible to male hormone as the result of a dominant mutation. When a hen-feathered breed is crossed to a normal race, the males in the F_1 are hen-feathered.

The Relative Absence of Sex Hormones in Insects.— Among insects hormones apparently play no important role in the development of secondary sexual traits. Instead, each cell develops its own secondary sex traits for the most part, as determined by the chromosomes which the cell contains rather than by hormones coming from the ovaries or testes. This can be proved by means of gynanders. For example, if in Drosophila the right foreleg were genetically male, it would develop the sex comb characteristic of the male even though the rest of the animal were female and contained an ovary. In moths, it is possible to produce artificial gynanders by grafting together, say, the front half of a male pupa and the hind half of a female. The resulting adult consists of a front half having the physical traits and instincts of a male, though the hind half is female and contains ovaries. But in man and other mammals, the ovary would produce hormones and these would be expected to influence any genetically male tissue in the same person, causing it to become female in appearance, at least partly. Thus there would be no gynander—no sharp division into male and female tissue. Gynanders have not been reported in man or any other mammal.

The Relation of Hormones to Sex Chromosomes.—It must not be thought that hormones are evidence against the chromosome mechanism of sex determination. The chromosomes determine whether the young embryo is to develop ovaries or testes; these then produce hormones and so influence the further development of sex (in vertebrates). Hormones represent just one step in the development of sex. There are numerous other steps leading up to the hormone stage and numerous after it, but the whole series

of steps is determined by what chromosomes the egg had at the time it was fertilized and by the environment in which the egg developed. It is true that hormones introduced from outside might influence sex development, as in the free-martin. This simply shows that the environment has an influence on sex development; it does not disprove the fact that normally sex is determined by chromosomes in man, Drosophila, and other organisms. It is also true that hormones sometimes fail to develop properly and that sex does not develop normally as a result. This may be due either to wrong heredity or wrong environment. It again does not disprove the existence of sex chromosomes and their role in sex determination.

The Problem of the Determination of Sex at Will.—If sex is ever to be determined at will in man, it will probably have to be done through the chromosome mechanism. It will be the father who will have to be experimented on, or the sperm cells derived from him, rather than the mother, for his sperm cells determine whether the child is to be a male or a female. Possibly some drug might be found that could injure one type of sperm or slow up its movements, leaving the other to fertilize the egg and produce the desired sex. It is conceivable that a hormone or drug might be found that could influence the sex development of the early embryo, regardless of its chromosomes, or even reverse its sex development. However, human beings whose sex development had been determined in this way would probably not be fully normal, and it would be better to attempt the control of the offspring's sex through the normal mechanism of determining sex, the chromosomes.

It is conceivable that mutations will be found which will control sex determination in man. In *Drosophila pseudo-obscura*, Dobzhansky and Sturtevant have found a mutation (sex linked) which destroys the Y chromosome at the reduction division and at the same time causes the equational division of the X, with the result that none of the sperm cells get a Y and all get an X. Therefore, males with this mutation produce only daughters, and the mutation is referred to as "sex ratio."

It seems not so very unlikely that we shall be able to determine sex at will some day, possibly in the near future. If so, there might be an excess of boys or of girls born according to the sex that happens to be in vogue. The scarcer sex would then automatically come into greater favor and in the following generation would tend to be produced in excess. In this way it is conceivable that the

controlled determination of sex may raise perplexing sociological problems which will have to be faced and solved.

SUMMARY

1. In man and many other forms of life there are two chromosomes which are of unequal size but which act as partners in the male. The larger is called the $X;$ the smaller the Y. In the female there are two X's. Thus the chromosomal formula of the sexes is $♀ = XX$, $♂ = XY$.

2. At the reduction division the two X's separate in the female and all the eggs get one X. In the male the X and Y separate, and half the sperm cells get an X, half a Y. At fertilization an egg might be fertilized by either an X- or a Y-containing sperm cell, giving XX and XY, or girls and boys in equal numbers (a 1 : 1 sex ratio).

3. No method is known of influencing the sex ratio in man, cattle, or other higher animals.

4. The X chromosome contains genes not directly concerned with sex, and in man a mutation in one of these caused color blindness. Hence the color-blind gene and its normal allele are inherited in the same way as the X chromosome. Other mutant genes are known to be in the X of man, Drosophila, and other animals.

5. There is no one primary gene for sex in the X chromosome, but many are scattered throughout the length of the X. This is shown by the fact that fragments from almost any part of the X will cause more complete female development when experimentally added (at the time of fertilization) to "intersexes" in Drosophila.

6. The Y chromosome in Drosophila is inert in the sense that it has very little influence on development. It does, however, contain about a half dozen genes necessary for the mobility of the sperm cells and one gene necessary for normal bristle development (the normal allele of "bobbed," also present in the X).

7. The inert material that makes up most of the Y is chromatin of a kind called "heterochromatin." This is to be contrasted to active chromatin or "euchromatin" (eu = good). During the resting stage and prophase of cell division the heterochromatin contains more nucleic acid than does the euchromatin, and during these stages, therefore, it stains more heavily with certain acid-staining dyes than does the euchromatin.

8. In some species the Y is very small or completely absent.

9. In man and Drosophila not only the X but also the autosomes contain genes necessary for sex development, and both the X and the autosomes must be present in the proper proportions for normal sex development. In the male these proportions are one X to two autosomes of each kind; in the female, two X's to two autosomes.

10. In chickens the female is XY, the male XX. This formula also applies to moths and some fishes.

11. In chickens a barred female (XY) transmits barred to her sons (XX) but not to her daughters. Hence the female must be XY. The inheritance of barred was known before the X and Y in chickens were identified under the microscope, and it showed by itself that the female must be the heterozygous sex (XY). This conclusion was later confirmed under the microscope. Thus the genetical and "cytological" findings were in agreement.

12. In the hymenoptera (ants, bees, and wasps) the females develop from fertilized eggs and are always diploid, but the males usually develop from unfertilized eggs and are haploid as a rule.

13. In wasps there is a series of alleles $(a, b, c,$ etc.) at a certain locus (the sex locus, really a small segment of a chromosome). Females are always heterozygous (as a/b, a/c or b/c); males are either haploid (as a, b, or c) or homozygous diploids (as a/a, b/b, or c/c). If a female and male are unrelated $(a/b \times c)$, all the fertilized eggs are heterozygous $(a/c, b/c)$ and therefore they develop into females. If the parents are related $(a/b \times a)$, half of the fertilized eggs are homozygous (a/a) and therefore they develop into males.

14. It is probable that the method of sex determination is the same in bees as in wasps. If queen bees are usually fertilized by unrelated males $(a/b \times c)$, then all the fertilized eggs are females $(a/c, b/c)$ and all the males are haploid.

15. In flowering plants with separate sexes the females are XX, the males XY. In Melandrium the Y takes an active part in sex determination, since plants without a Y are never males.

16. In the marine worm *Bonellia* the females are fully developed but the males are very small and attached to the females. A larva develops into a male if it becomes attached to an adult female, but otherwise it develops into a female. The environment therefore determines sex in Bonellia.

17. Of the various possible methods of sex determination, the one usual for animals is that found in man and Drosophila, in which sex development depends on the proportions of the X to the autosomes, there being one proportion for normal males, another for normal females.

18. "Intersexes" are individuals that are intermediate between males and females.

19. In Drosophila, intersexes contain two X's and three sets of autosomes. This ratio $(2\ X : 3\ A)$ is intermediate between that normal for males $(1\ X : 2\ A)$ and that normal for females $(2\ X : 2\ A)$, and it is the cause of intersexes in Drosophila.

20. In the gypsy moth, intersexes are caused by crossing geographical races differing in the "strength" of determiners concerned with sex. Thus when a European female (weak) is crossed to a Japanese male (strong), the female offspring develop male traits. They have the sex formula normal for female moths (XY) and they have the normal number of autosomes (two sets), so they are not caused by the wrong ratio of X to autosomes. Their genetic constitution is not known.

21. In pigs, females are XX and males XY, but intersex males (with the formula XY) are produced by a mutation which perhaps strengthens the sex genes in the X so that 1 X approaches 2 X's in strength, or which perhaps weakens the sex genes in the autosomes, thus making the X relatively stronger as compared to the autosomes.

22. By "sex reversal" is meant the complete change of one sex into the other, so that the individual now has the chromosome formula of the one sex but the appearance of the other.

23. In the fish *Lebistes*, the female is normally XX and the male XY. The X contains predominantly genes for femaleness and the Y genes for maleness, but at many autosomal loci the fish is heterozygous for male and female genes, and by rare Mendelian recombination, an XX fish might come to have an unusually large number of male genes in its autosomes as compared with normal, so that it develops into a male despite the fact that it has the chromosome formula of the female (XX).

24. In corn, barren (ba) makes the silks sterile and tassel-seed (ts) changes the male organs (tassels) into female (or seed-bearing) organs. It also prevents ba from having any effect. A plant of genotype $\dfrac{ba\ ts.}{ba\ ts}$ is a female, one of genotype $\dfrac{ba\ +}{ba\ ts}$ is a male, and when the two are bred together, they produce females and males in equal numbers, thus: $\dfrac{ba\ ts}{ba\ ts}$ (♀)

$\times \dfrac{ba\ +}{ba\ ts}$ (♂) $\rightarrow 1\ \dfrac{ba\ ts}{ba\ ts}$ (♀) $: 1\ \dfrac{ba\ +}{ba\ ts}$ (♂).

25. A "gynander" is an individual in which the tissues are genetically partly male and partly female, as when the left half of the body has the chromosomal formula of a male, the right half that of a female. Gynanders are known in Drosophila, silkworms, and bees.

26. In Drosophila a gynander is produced by the loss of an X at a mitotic cell division in the development of an XX egg. One of the products of the division thus has only one X and develops into male tissue, the rest of the body being female (XX).

27. In silkworms (in which the females are XY), an egg sometimes fails to extrude a polar body and therefore contains two nuclei, one with an X

and one with a Y. Such an egg might be fertilized by two sperm cells (both X-containing) and so give rise to XX tissue (male) and XY tissue (female).

28. In bees, a binucleate egg might give rise to a gynander if one nucleus was fertilized and the other not, since the fertilized nucleus would give rise to female tissue and the unfertilized one to male tissue.

29. Hormones influence the development of sex, but hormones themselves are the products of genes.

30. It seems likely that sometime in the future it will be possible to have boys or girls at will through a knowledge of sex determination.

PROBLEMS

1. A color-blind man has a normal brother and a color-blind sister. Give the genotypes of the parents.

2. In Drosophila the mutant gene for light eye color known as "vermilion" (v) is in the X chromosome and is recessive to red $(+)$. A vermilion female is crossed to a red-eyed male. Give the eye color of the F_1 (together with their sex) and of the F_2 (when the F_1 are interbred).

3. In chickens a recessive mutation in the X changed brown feathers $(+)$ to silver (s). A brown female is crossed to a silver male. Give the color of the F_1 (together with their sex) and of the F_2 (when the F_1 are interbred). Remember that in chickens the female is XY, the male XX.

4. It is ordinarily difficult but often desirable to tell the sex of newly hatched chicks. Tell how we might cross a barred race of chickens by a black so as to identify the sex of the newly hatched chicks from their feather color (which is distinct at the time of hatching).

5. A male chicken contains a recessive lethal in one of his X chromosomes (l) and the normal allele $(+)$ in the other. If he is bred to any female, what will be the expected sex ratio among the offspring?

6. In chickens Crew reported a case of sex reversal in which a female changed over to a male. Assuming that this chicken, now male, still retained the chromosomal formula of a female, what would be the expected sex ratio among his offspring if he were bred to a normal female?

7. In Drosophila there is a race of flies, discovered by Bridges, in which the females contain a Y in addition to two X's. As a rule the two X's separate (or disjoin) at the reduction division, so that an egg usually gets one X (with or without the Y), but sometimes the two X's go to one pole (referred to as "non-disjunction" of the X's) and the Y to the other pole, with the result that a certain proportion of the eggs get two X's.

A vermilion-eyed female of non-disjunctional stock is crossed to a red-eyed male (vermilion being a recessive mutation in the X). Give the eye color and sex of the F_1 offspring derived (a) from the normal eggs, (b)

from the 2 X eggs fertilized by Y sperm, (c) the no-X eggs (with a Y) fertilized by X sperm.

8. A white-eyed Drosophila is crossed to a red-eyed male (white being a recessive mutation in the X). What eye colors would the F_1 ordinarily be expected to have? Suppose some of the F_1 females have white eyes (exceptional females), and some of the males have red eyes (exceptional males). Account for the exceptional offspring.

9. In the fish *Aplocheilus* the female is XX, the male XY. The Y in this fish (and in fishes generally) is not inert as in Drosophila; it contains normal alleles, like those of the X, and is for the most part homologous with the X. Aplocheilus is normally brown. Blue (b) is a recessive in the X, red (r) a recessive in an autosome. Thus if we designate the normal alleles as $+$ and a normal allele in the X as $+(X)$, a normal (brown) female is $\dfrac{+(X)\ +}{+(X)\ +}$; a blue female is $\dfrac{b(X)\ +}{b(X)\ +}$; a red, $\dfrac{+(X)\ r}{+(X)\ r}$. The combination $\dfrac{b(X)r}{b(X)r}$ is white. In the cross below, the Y chromosome always contains the normal allele of b, and therefore a male is never blue, nor is he ever white (that is, blue red). Let us designate the normal allele in the Y as $+(Y)$.

A white female is crossed to a pure brown male, and the F_1 are bred together. Give the genotypes and phenotypes in the F_1. Derive the F_2 by the branching method. First derive the F_2 genotypic classes for the b locus only, giving the females first, then subdivide each of these classes on the basis of the r locus, but give the abbreviated genotypes for this locus ($\frac{3}{4}\ +$ and $\frac{1}{4}\ r$), and finally give the phenotypes. Do the same for the males.

10. In bees a sperm cell contains 16 chromosomes and so does an egg. The females develop from fertilized eggs and the males from unfertilized eggs. Give the number of chromosomes in the body cells of each sex. Tell also in which sex a normal reduction division would of necessity take place, and in which sex it would be omitted.

11. Assume that a female Habrobracon is heterozygous for genes c and d at the sex locus (or c/d). Assume also that 80 per cent of the eggs she lays are fertilized (the rest being unfertilized). Give the sex ratio among her offspring if the male parent is (a) related ($c/d\ ♀ \times c\ ♂$), (b) unrelated ($c/d\ ♀ \times e\ ♂$). Tell what proportion of the males are biparental in each case.

12. In Habrobracon orange eyes (o) are recessive to black ($+$). An orange female is crossed to a haploid black male, and 80 per cent of the eggs are fertilized. Give the F_1 classes of offspring and their expected ratio if the male parent is (a) unrelated to the female parent, (b) related.

13. Suppose a mutation should take place in the Y chromosome of man and that this mutation should cause the degeneration or loss of the X at the reduction division in the male. Assume further that we begin with one male with the mutation in question, that he has only two children, and that all his descendants have two children each but no more (two children per man and wife not resulting in any increase in the population). What would be the sex of his two children? Of his four grandchildren? Of his eight great-grandchildren? What effect would this mutation be expected to have eventually on the sex of the entire population?

7. *INBREEDING AND OUTBREEDING*

B Y INBREEDING we mean the production of offspring by closely related parents; by outbreeding, their production by unrelated parents. There are various degrees of inbreeding and outbreeding. Brother-and-sister matings are very close inbreeding, first-cousin matings less close.

Is Inbreeding Harmful?—We have always heard that blood relatives are apt to have defective children. This view has been held since the earliest historical times and today there are laws in all civilized countries against the marriage of very close relatives. It has been in fact observed that the children are sometimes defective when close relatives marry, even though both parents are apparently normal. In small communities, where there is apt to be a good deal of inbreeding, idiot children are born more frequently than in cities.

If we look to the lower forms of life, we seem to get some encouragement for the conventional opposition to inbreeding. The closest form of inbreeding is self-fertilization, possible in the case of hermaphrodites. But often we find adaptations against self-fertilization, such as the ripening of the sperm and egg cells at different times, or special devices for securing cross-pollination in plants. Moreover, when corn is grown it is the practice of the grower to pollinate the seed-bearing plants with pollen from different plants rather than with their own. If the plants are allowed to self-pollinate they produce inferior seeds, many of them growing into small and defective plants. All this looks like a good case against inbreeding.

There is, however, something to be said on the other side. Hermaphrodites—especially plant hermaphrodites—do not always outbreed. That whole family of plants to which the bean and pea belong habitually self-pollinate. Still these plants are as vigor-

ous as any in the plant kingdom. There are also the practices of animal breeders to consider. The best breeds of horses, dogs, and other domesticated animals are usually closely inbred, a practice which the breeder refers to as "line breeding." The breeder of a thoroughbred race of horses knows that he would lower the quality of his highly selected stock by introducing into it the blood of some outside stock. Thus it appears that inbreeding is not bad after all and the whole situation becomes very confusing.

The Mendelian Explanation of the Effects of Inbreeding. —One of the practical results of Mendel's principle has been to offer a final solution to this ancient problem of inbreeding. We might consider specifically the Mendelian explanation of the occasional appearance of idiocy among the children of closely related parents. It will be recalled that there is an hereditary form of idiocy which probably arose by the mutation of one of the genes for normal mentality, and that the mutated gene (i) is recessive to the normal gene ($+$) from which it arose. Two people might apparently be normal, yet both might be hybrid for idiocy ($i/+$). The mating of the two hybrids ($i/+ \times i/+$) would yield offspring in the simple Mendelian ratio of 1 $+/+$ (pure normal) : 2 $i/+$ (hybrid normal) : 1 i/i (idiot), or 3 normals : 1 idiot.

Now normal parents can have idiot offspring (of the hereditary kind above mentioned) only in case both parents are hybrids. This fact holds regardless of whether or not the parents are close relatives. In the case of brother and sister matings, however, the chances are comparatively great that if one parent is a hybrid, the other one is also. From the fact that brother and sister have the same parents, there is a likelihood that if either one received the gene for idiocy from his father or mother, the other one also did. In the case of first-cousin marriages, the chances are also fairly high that if one parent is a hybrid, the other also is, for first cousins have two grandparents in common and they might therefore have a given kind of gene in common.

But suppose that a hybrid, say, the man above mentioned, mated with an unrelated person. The chances now are that any normal-appearing woman with whom he mated would not be another hybrid as before, when his sister or other close relative was considered, but rather that she would be a pure normal. Persons hybrid for this particular gene are not nearly so common in the general population as are pure normals, and any outside person

with whom the hybrid mates and who appears normal is much more apt to be a pure normal than is a close relative of the hybrid. To put the thing in symbols the mating would now in all probability be $i/+$ (hybrid normal) $\times$ $+/+$ (pure normal). This mating gives only normal-appearing offspring in the ratio of $1 +/+ : 1$ $i/+$. Note, however, that half the offspring are hybrids, and that the gene i is still in existence. The gene cannot be made to disappear by outbreeding. It is simply under cover, so to speak. Note also that inbreeding does not cause the production of the gene for idiocy but that it simply allows two such genes to come together and to express themselves.

Inbreeding does not allow only bad qualities to express themselves in the offspring. Any good qualities that are dependent upon recessive genes would come to the surface in the same way as do the bad qualities.

If a recessive gene were very common in the general population, such as the gene for blue eyes as opposed to brown, any two persons selected at random might very well both be hybrid for the gene, regardless of whether they were related or unrelated. In a case of this kind outbreeding would not necessarily prevent the recessive gene from expressing itself. It is only when a gene is comparatively *rare* in a population that a non-relative of a given hybrid is more likely to be a pure dominant than a hybrid. Only then will outbreeding prevent the appearance of the trait which depends on the recessive gene in question, for now all the children will receive the dominant from their pure parent.

We can then summarize as follows. When a recessive gene is comparatively rare in the general population, inbreeding allows two such genes to come together and express themselves, but outbreeding does not. The reason is that a relative of a given hybrid is much more likely also to be hybrid than is a non-relative of the hybrid in question, and two recessive genes can then come together and express themselves.

The Non-effect of Inbreeding on Genes Proper.—It might perhaps seem that inbreeding allowed bad recessive genes to become more numerous than if outbreeding took place. But neither inbreeding nor outbreeding *in themselves* have any influence on the per cent of recessive genes in the entire population. In order to show this we can, for the sake of simplicity, deal with a population which consists of just two families, one (family a) with two chil-

dren, both hybrids for idiocy $(i/+)$ and the other (family b) also
with two children but both normals $(+/+)$. First let the children
of each family inbreed $(i/+ \times i/+$ and $+/+ \times +/+)$. Second,
let the two members of family a $(i/+$ and $i/+)$ mate with the
two members of family b $(+/+$ and $+/+)$ giving us two matings,
both $i/+ \times +/+$. Also, let each mating produce four children.
These matings and their average results are shown below.

Inbreeding

Family a $i/+ \times i/+$ gives $1 +/+ : 2\ i/+ : 1\ i/i$
Family b $+/+ \times +/+$ gives $4 +/+$ (if there are four children)

Total i's = 2 in 8, or 25%   Total i's = 4 in 16, or 25% (no
                                    change in per cent)

Outbreeding

Family a Family b Children
    $i/+ \times +/+$ gives $2\ i/+ : 2 +/+$
    $i/+ \times +/+$ gives $2\ i/+ : 2 +/+$

Total i's = 2 in 8, or 25%   Total i's = 4 in 16, or 25% (no
                                    change in per cent)

In the above population we begin with 25 per cent of i genes and
end with 25 per cent, both in the case of inbreeding and outbreed-
ing. Thus the per cent of i genes in the entire population has not
been changed by either inbreeding or outbreeding. The same
thing would hold in a larger population.

**The Elimination of Hybrids from a Population Through
Inbreeding.**—Inbreeding tends to eliminate hybrids from a popu-
lation and to substitute the pure types for them. Just how this
elimination of hybrids comes about through inbreeding can be
shown best in connection with hermaphroditic plants where self-
fertilization, the closest form of inbreeding, may take place. Thus
suppose that we began with some convenient number of F_1 hybrid
plants, say, 800 pink four-o'clocks (which are hybrid for white and
its normal red allele, or $w/+$). In each generation let one plant
reproduce just one offspring so as to keep the number of plants
constant (800). The F_1 hybrids will produce F_2 offspring in the
ratio of $1 +/+$ (red) : $2\ w/+$ (pink) : $1\ w/w$ (white). That is to
say, on the average $\frac{1}{4}$ of the offspring, or 200, will be $+/+$ (red),

$\frac{2}{4}$ or 400 will be $w/+$ (pink), and $\frac{1}{4}$ or 200 will be w/w (white). The hybrids now form only 50 per cent of the total population. When the F_2 reds and the whites reproduce they maintain their numbers (at 200 each) but when the 400 hybrids reproduce they again throw offspring in the 1 : 2 : 1 ratio, or an average of 100 $+/+$ (red) : 200 $w/+$ (pink) : 100 w/w (white). Now only 200 of the total 800 offspring are hybrids, or 25 per cent. The proportion of hybrids has gone from 100 per cent in the F_1 to 50 per cent in the F_2 to 25 per cent in the F_3. In the F_4 the proportion would be $12\frac{1}{2}$ per cent. These results are summarized in Table 2 below.

TABLE 2.   REDUCTION IN PROPORTION OF HYBRIDS IN A POPULATION AS THE RESULT OF SELF-FERTILIZATION

Generation	Composition of Population			Hybrids	
	Reds $(+/+)$	Pinks $(w/+)$	Whites (w/w)	Number	Per Cent
F_1		800 $w/+$		800	100
F_2	200 $+/+$	400 $w/+$	200 w/w	400	50
F_3	200 $+/+$ 100 $+/+$	200 $w/+$	100 w/w 200 w/w	200	25

Each generation of self-fertilization reduces the proportion of hybrids by a half. After ten generations of self-fertilization the proportion of hybrids in the population would have been reduced to about 1 in 1,000, and for all practical purposes the hybrids would have been eliminated. The population would now consist almost exclusively of the two pure classes $+/+$ (red) and w/w (white).

Pure Lines.—If a population of peas were hybrid for red and white flower color, continued self-fertilization would make it consist eventually of pure reds and pure whites. If it had in addition

been hybrid for tall and dwarf at the start, it would eventually consist of pure talls and pure dwarfs. But the reds might be either tall or dwarf; so might the whites. Thus the population would eventually consist of four homozygous classes: (1) red tall, (2) red dwarf, (3) white tall, (4) white dwarf. If we let w and d stand for white and dwarf, respectively, and $+$ signs for their normal alleles, then the genotype of the original hybrid population was $\dfrac{w}{+}\dfrac{d}{+}$. But after continued self-fertilization the population consists of four pure genotypes: (1) $\dfrac{+}{+}\dfrac{+}{+}$ (red tall), (2) $\dfrac{+}{+}\dfrac{d}{d}$ (red dwarf), (3) $\dfrac{w}{w}\dfrac{+}{+}$ (white tall), and (4) $\dfrac{w}{w}\dfrac{d}{d}$ (white dwarf).

If the original population had been hybrid for still another pair of alleles, say, green seed color and its normal allele $(g/+)$, then each of the four classes of offspring listed above would have become pure for either $+$ or g, giving us eight pure classes in all. Any additional pairs of alleles for which the original F_1 were hybrid would in a similar manner become sorted out into plants pure for either allele, and the number of pure classes would be correspondingly increased.

The various pure races produced after a period of self-fertilization are known as *pure lines*. Once these have come into existence, each one continues to reproduce only its own genotype when it is self-fertilized.

Heterosis, or Hybrid Vigor.—Hybrids are often more vigorous than their parents, as is well shown in corn. When two distinct strains of corn are crossed the offspring are as a rule much taller, more vigorous and fertile than either parent (Fig. 52).

However, outbreeding does not produce increased vigor because of some peculiar effect that follows upon a mixing of "bloods," for vigor has a definite, though complicated, genetic basis. In plants vigor depends on a big system of roots, on well-developed leaves with plenty of green coloring substance, on stout stems, and on many other things. Vigor has a correspondingly complicated genetic basis and is dependent on many genes. Moreover, vigor as a rule depends on dominant genes. We can label these genes A, B, C, etc., and the corresponding weak genes a, b, c, etc. Let us limit ourselves to just two pairs of genes concerned with vigor. There

Fig. 52. Hybrid vigor in corn. The two outer rows (of those with the white stakes in front of them) are dwarf races and are the parents of the middle row. (From D. F. Jones.)

might be two pure strains of plants, one of which had the genes Ab, the other aB. Each would be weak because neither has both A and B. Now we cross the two strains, and we produce hybrids $\left(\dfrac{A\ b}{a\ B}\right)$. These receive A from one parent, B from the other. They also receive the weak genes a and b, but these are recessive and do not influence the development of the offspring in the presence of the corresponding dominants A and B. The hybrids accordingly develop into plants that are stronger than either parent. Had we been dealing with a larger number of genes the result would have been the same, provided each parent had contributed to the offspring some dominant genes that the other parent lacked. This increased vigor produced by crossing two different strains or races is known as *heterosis*.

The crossing of two distinct strains does not always cause an increase in vigor. When one of the strains has all the dominant genes $\left(\dfrac{A\ B}{A\ B}\right)$ and the other none $\left(\dfrac{a\ b}{a\ b}\right)$, then the hybrid offspring $\left(\dfrac{A\ B}{a\ b}\right)$ are no stronger than the parent with the dominants. The offspring are stronger than both parents only when they have more kinds of dominant genes than either parent, as when the parents are $\dfrac{A\ b}{A\ b}$ and $\dfrac{a\ B}{a\ B}$ and the offspring $\dfrac{A\ b}{a\ B}$. Increased vigor of hybrids is not due to heterozygosis in itself, as the term heterosis might suggest, but simply to the fact that the hybrid offspring as a rule contain dominant alleles for vigor at more loci than does either parent by itself.

It has been known for a long time that the crossing of distantly related strains causes an increase of vigor in the offspring as compared to the parents. We can readily understand now how the element of distant relationship enters into the situation. Two strains that are distantly related have had a different history for a comparatively long time, and each is likely to contain mutant genes which the other lacks, some of them dominants. When the two strains are crossed the offspring receive the combined dominants of the two and so are more vigorous.

In summary, the good effects of outbreeding on vigor are probably due entirely to the mutually complementary manner in which

the parents supply dominant genes to the hybrid offspring. However, this does not explain why strong traits are as a rule dependent upon genes that are dominant rather than recessive.

The Drop in Average Vigor When Outbreeding Is Followed by Inbreeding.—Consider now what would happen to the average vigor of a stock if it started to inbreed after having outbred. The stock with which we begin would be hybrid because it has been produced by outbreeding. Thus if the parents which produced this stock were $\dfrac{a\ B}{a\ B}$ and $\dfrac{A\ b}{A\ b}$, the stock in question would be $\dfrac{a\ B}{A\ b}$. It would have the vigor that usually comes with hybridity. But if the stock were now inbred over a number of generations it would eventually become homozygous and consist of four pure lines; namely, $\dfrac{A\ B}{A\ B}, \dfrac{a\ B}{a\ B}, \dfrac{A\ b}{A\ b}$, and $\dfrac{a\ b}{a\ b}$. The first of these $\left(\dfrac{A\ B}{A\ B}\right)$ would be just as vigorous as the original F_1 hybrids $\left(\dfrac{a\ B}{A\ b}\right)$. But the rest would be weaker because they do not contain both A and B. Therefore the *average* vigor of the population would be lowered.

The drop in vigor would be more rapid in the earlier generations of inbreeding than in the later, because of the fact that in the first generation the absolute drop in per cent of plants hybrid at a given locus is 50, in the second it is only 25, etc. This sort of thing has been observed in corn. After a population has been sorted out into pure lines, then inbreeding produces no further decline in vigor.

A Practical Difficulty in Getting Pure Lines with Maximum Vigor.—It is customary to get seed for raising corn by crossing different strains of corn. The particular combination of strains used as parents produces very vigorous offspring because of heterosis. Theoretically we might by continued inbreeding produce a line that was pure for all the dominant alleles and that was just as vigorous as the hybrid, but there are practical difficulties in the way of this. Very many genes influence vigor in corn, not just two as in the theoretical example given above. Hundreds and possibly thousands of pure lines might have to be produced in order to get the one that had all the dominant genes found in the F_1 hybrid. Moreover, a given chromosome might contain a dominant gene for vigor at one locus but a weak recessive at another closely neigh-

boring locus. When genes are in the same chromosome, and especially when they are close together, they tend to remain together in heredity. It is therefore sometimes difficult to get by inbreeding a combination which is pure for two dominant alleles at different loci in the same pair of chromosomes—much more difficult than when the genes are in different pairs of chromosomes. It is easier in practice to produce the hybrid strain anew each generation than to attempt to get a pure line which contains all the dominant alleles.

The Explanation of the Apparently Conflicting Effects of Inbreeding on Vigor.—The apparently conflicting effects of inbreeding on the vigor of the offspring can now be understood. Animals or plants which outbreed are hybrid to a certain extent. Close inbreeding on their part is therefore followed by a reduction in the average vigor of their descendants. But plants which habitually self-fertilize consist of pure lines and inbreeding has no effect on the vigor of their offspring. Such is the case with the various members of the pea family. These inbreeding races have come by the process of natural selection to have only genes for strong traits; the weaker types have been eliminated in competition with the stronger. It is for this reason that they are vigorous; and it is because the process of inbreeding has made them pure that further inbreeding can have no effect whatever on their vigor.

The various highly selected races of horses and other domesticated animals are not so very adversely affected by inbreeding because they, too, have become comparatively pure by the very process of inbreeding. These races at the same time possess a highly selected lot of genes and the only effect of breeding to outside races would be an unfavorable one, from the standpoint of the breeder.

The Relation of Inbreeding and Outbreeding to Natural Selection.—Inbreeding, we saw, allows recessives to come to the surface, as when two cousins, hybrid for idiocy, marry and have an idiot child. In a state of nature the idiot would die in competition with the normals before he reached reproductive age. Along with him the gene for idiocy would die and so this gene would gradually be eliminated from the population. Thus inbreeding *assists* natural selection by the bringing of weak recessives to the surface.

Outbreeding would do just the opposite. For outbreeding produces hybrids, and in a hybrid a recessive gene is prevented from expressing itself by its dominant allele. The hybrid is therefore

neither hampered nor helped by any recessive genes for which it is hybrid. For instance, a person hybrid for idiocy develops into a normal person and he is not selectively eliminated in any struggle with the pure normals. Hence his idiot gene is not eliminated. It continues to be protected against the influence of natural selection in any of the offspring that are hybrid and in all future descendants that are.

Chromosome Degeneration Through Continuous Hetero-zygosity.—The Y chromosome in a good many species has undergone degeneration. The reason for this change is simple enough in view of what has just been said. Recessive mutations that occur in the Y are prevented from expressing themselves by their dominant normal alleles in the X. Therefore weak recessives in the Y do not handicap the animal or plant that carries them, and so they do not come under the influence of natural selection. As a result the Y chromosome has gradually deteriorated through the accumulation in it of weak and inert genes.

With the X, however, it is different. The heterozygous sex transmits its X to the homozygous sex in the next generation, as for example when the male in Drosophila (XY) transmits his X to his daughters (XX). Weak recessive genes in the X would express themselves in any members of the homozygous sex which carried the weak genes in each of its X's and so would be eliminated through the action of natural selection. This keeps the X up to par. Moreover, once the Y has degenerated, it no longer contains dominant alleles, and so it does not prevent the expression of any weak recessives in the X in the heterozygous sex. This further keeps the X up to par.

The Comparative Merits of Inbreeding and Outbreeding.—We are now prepared to pass judgment on the relative merits of inbreeding and outbreeding. Inbreeding allows natural selection to operate on recessive genes; outbreeding does not. But inbreeding does not allow for the introduction of good mutations from outside strains. This is especially true if inbreeding is continued generation after generation. It is here that outbreeding has the advantage over inbreeding. For, two distinct strains may each possess some good qualities that the other lacks, and by crossing and Mendelian recombination the good qualities of both parents can be brought together in the F_2 and later generations. In this way all the good mutations that arise among the scattered members of a race can be

concentrated into a single line. It is for this reason that most people today, and most members of any other species, contain practically all the good mutations that have arisen in the evolutionary history of the race.

We see, then, that both inbreeding and outbreeding by themselves have an advantage and a disadvantage. For the speedy evolution of a race a combination of the two methods is really the most desirable; namely, inbreeding for the most part interrupted by occasional outbreeding. In this way recessive mutations can come under the influence of natural selection (by means of inbreeding), and at the same time good mutations can from time to time be introduced into a strain from the outside (by means of outbreeding).

In the pea family is realized the very method of breeding that is most advantageous for a species. For peas as a rule shed their pollen before the flower opens and so undergo the closest form of inbreeding—self-fertilization. But insects carry pollen from one flower to another and occasionally bring about the cross-pollination of a flower which perhaps was somewhat late in shedding its pollen.

Bees and other Hymenoptera have evolved a unique method of breeding that permits of the advantages of both inbreeding and outbreeding. It will be recalled that the unfertilized eggs of bees, as well as the fertilized eggs, can develop. The fertilized eggs of course receive their chromosomes from a double source—from both the male and the female parent—and they make possible to the species the advantages of outbreeding. The unfertilized eggs on the other hand have just a single set of chromosomes—their own set. When they develop without fertilization any recessives that they contain express themselves, since there are no dominant alleles to prevent them from doing so. The recessives therefore are subject to the influence of natural selection. Thus the species derives the advantages of outbreeding through its fertilized eggs and the advantages of inbreeding through its unfertilized eggs. We find substantially the same double method of reproduction in certain other forms of life in which reproduction during the summer and spring may take place through unfertilized eggs (parthenogenetically), followed by regular sexual reproduction and fertilized eggs in the late fall. These are the eggs which winter-over and hatch in the spring.

In most species of animals and plants there occurs a combination of inbreeding and outbreeding. The two methods of breeding are not mutually exclusive but may take place at the same time. When a person marries, say, a second or third cousin, he is inbreeding to a certain extent and at the same time outbreeding. Even if he should marry a woman whom he regarded as strictly unrelated, he would often find upon examining his genealogy and hers that both had some ancestors in common and that they were related, though perhaps distantly.

The Difference in Degree of Inbreeding in Small and Large Communities.—The question then arises, "To what extent is the human race inbreeding at present?" To answer this question we have to consider separately small communities and large, for the amount of inbreeding varies with the size of the community. In almost any small village there is a considerable amount of inbreeding, especially if there is no shifting of the population. This follows from the fact that a person doubles the possible number of his ancestors for each generation that he goes back, and a person living in a small stable community could not have the full possible number of ancestors for very many generations before the figure equaled the total number of people in his community. In a large community, on the other hand, especially one with a shifting population, there is comparatively little close inbreeding. The close relatives of a person in the population at large are greatly outnumbered by the more distantly related, and the chances that he will marry a close relative are much less than in a small community.

In brief, there is rather close inbreeding in small villages; in large cities there is more outbreeding. Accordingly there is a relatively higher proportion of idiots in small villages as compared with cities. It does not follow, however, that the proportion of *genes* for idiocy is any greater in the one than in the other. For in large cities these genes are simply scattered among hybrids for the most part. In villages they are concentrated or rather segregated among the idiots. But there is greater likelihood that a normal-appearing person in a large city is actually hybrid for idiocy than one in a village and that he is potentially the parent of idiot children.

The proportion of recessive traits in general whether good or bad would be expected to be greater in villages than in cities. In

villages there should be a relatively high proportion of persons with exceptional ability of a kind that is dependent upon recessive genes. But persons of ability often migrate to the larger cities and carry away the good genes from the country.

Many species are divided into groups that are more or less stable, as herds of cattle, flocks of birds, schools of fish, and fields of flowers. If the group is small, inbreeding takes place in it just as it does in a small human community. Even animals that do not form groups such as cats and bears are limited in their wanderings. It is possible that in the many instances when animals do not wander very far from their place of birth brother and sister matings or matings between parent and offspring sometimes occur.

Just to what extent very close inbreeding has taken place in the early history of the human race is difficult to determine. But primitive man and his pre-human ancestors almost certainly lived at one time or another in very small isolated groups, groups which may not, in fact, have been any larger than the family itself and in which close inbreeding must have occurred. With the introduction of modern methods of transportation accompanied by the intermingling of peoples and the growth of cities, the balance has been thrown in favor of outbreeding.

As the result of outbreeding the human race is hybrid to a large extent. Hence there is always a good deal of genetic variation among the children in the same family. This would not be true if there were any such thing as a pure "Nordic" type, or any other pure type among human beings.

Practical Applications of the Mendelian Analysis of Inbreeding.—So much for the Mendelian analysis of inbreeding and outbreeding. What practical application can be made of it? There are first our domesticated races of animals and plants to consider. It should be said to the breeder's credit that he has often anticipated theory in a practical way. To improve the qualities of a race for human needs, theory indicates that one might first cross it to some outside stock in order to bring together the good qualities of the two stocks. This, however, also brings together their bad qualities. If the cross is followed by inbreeding, the good qualities can be segregated from the bad and then the good can be selected and the bad rejected.

But often the breeder is working under great limitations. It would be very expensive and time-consuming to cross two breeds

of horses or cows and then inbreed their progeny in large enough numbers and over a large enough number of generations to segregate out and select the good qualities of the two races. The breeder has found it more practical in the case of horses and cows to limit himself to inbreeding his best stocks and rigorously selecting the best that turns up. He at least maintains the high quality of his stocks in this way, and with patience and close application he occasionally finds something new and better, representing undoubtedly a mutation. In the case of plants which do not take long to grow up from seeds and which can be grown in larger numbers without great expense, it is practical to cross, then inbreed and select over a large number of generations, and to work with a large number of plants. All this the breeder has done.

What practical suggestions can the Mendelian specialist give to the person who wants to marry a first cousin and who comes to him for advice? Shall he approve of it? The answer depends largely upon a number of things. If examination of the man's family history shows that there is no gene for some seriously objectionable trait in the family, then there can be no strong objection to the marriage. In the case of the Darwin family with its exceptionally good genes the marriage of cousins was very desirable. But when there is some bad recessive gene in a family there is danger that first cousins might both be carrying it, as hybrids, and that some of the children from their union might be defective. In this case it would be undesirable from their own personal standpoint that they should marry, but from the standpoint of the race it would be preferable that they should marry each other rather than outsiders if they are going to have children. For by marrying outsiders they simply allow the bad gene to survive under the protection of its dominant allele and to become scattered among the population at large. But if they intermarry they force the bad gene to come from under cover, and if society now sees fit, it can eliminate the bad gene by preventing the reproduction of the defectives who carry it. No brutal measures would be necessary on the part of society to achieve its end. The defectives could be sterilized by a method which would in no way affect the general health or character of the individual, by cutting or tying the ducts of the reproductive organs.

Whether or not the Mendelian solution of the inbreeding problem will have further practical applications remains for the future to

decide. For the present it will at least be evident that Mendel's discovery has put an end to the confusion of ideas that previously existed on the subject.

SUMMARY

1. Close inbreeding does not produce defective genes. It merely allows the coming together of defective genes.

2. Inbreeding does not cause any spreading of defective genes in a population; neither does outbreeding in itself prevent their spread.

3. Close inbreeding causes the elimination of hybrids from a population. Thus if we began (in the F_1) with a field of 800 pink four-o'clocks ($w/+$) and allowed them to reproduce in each generation by self-fertilization, keeping the total number at 800 in each generation, then in the F_2 there would on the average be 200 $+/+$ (red) : 400 $w/+$ (pink) : 200 w/w (white), so that the hybrids would now form only 50 per cent of the total. The 400 $w/+$ would in the F_3 produce 100 $+/+$ (red) : 200 $w/+$ (pink) : 100 w/w (white), and so the hybrids would now form only 25 per cent of the total population (200 in 800). Thus in each generation the proportion of hybrids would be reduced to half, and in about 10 generations they would have almost disappeared, leaving only the pure types ($+/+$ or red and w/w or white).

4. If we started with a field of yellow round peas that were hybrid for green and wrinkled peas $\left(\dfrac{g}{+} \dfrac{w}{+} \right)$, and allowed it to reproduce by self-fertilization, then the field would eventually consist of pure yellow ($+/+$) and pure greens (g/g), and each of these classes would consist of pure rounds ($+/+$) and pure wrinkled (w/w), giving us four pure classes in all: $\dfrac{+}{+} \dfrac{+}{+}$ (yellow round), $\dfrac{+}{+} \dfrac{w}{w}$ (yellow wrinkled), $\dfrac{g}{g} \dfrac{+}{+}$ (green round), and $\dfrac{g}{g} \dfrac{w}{w}$ (green wrinkled).

5. Races that are pure as a result of inbreeding are known as *pure lines*.

6. If two races are crossed, each pure for a different weak recessive $\left(\dfrac{a}{a} \dfrac{B}{B} \times \dfrac{A}{A} \dfrac{b}{b} \right)$, then the offspring contain both dominant alleles $\left(\dfrac{a}{A} \dfrac{B}{b} \right)$ and they therefore do not show the effect of either pure recessive. If the dominants make for vigor (as is often the case), then the F_1 are more vigorous than either parent.

7. The increased vigor, often seen in hybrid offspring, is known as *heterosis*.

8. If one parent had both dominant genes and the other neither $\left(\dfrac{A}{A}\dfrac{B}{B}\times\right.$ $\left.\dfrac{a}{a}\dfrac{b}{b}\right)$, then the F_1 $\left(\dfrac{a}{A}\dfrac{b}{B}\right)$ would be no more vigorous than the parent that was pure for both dominants $\left(\dfrac{A}{A}\dfrac{B}{B}\right)$. Hence, hybridity in itself does not produce increased vigor.

9. Heterosis is due to the bringing together of dominant alleles which have a complementary influence on vigor (as when A and B, previously separate, are brought together).

10. If we begin with a field of hybrid plants $\left(\dfrac{a}{A}\dfrac{b}{B}\right)$ and reproduce them by self-fertilization, then we eventually get four pure lines $\left(\dfrac{A}{A}\dfrac{B}{B},\dfrac{A}{A}\dfrac{b}{b},\right.$ $\dfrac{a}{a}\dfrac{B}{B}$, and $\left.\dfrac{a}{a}\dfrac{b}{b}\right)$. Only one of these $\left(\dfrac{A}{A}\dfrac{B}{B}\right)$ contains both dominants and is as vigorous as the original hybrid $\left(\dfrac{a}{A}\dfrac{b}{B}\right)$. Hence, there is a drop in the *average* vigor of the population with inbreeding.

11. Races which outbreed are ordinarily hybrid. Inbreeding in such races is followed by a drop in average vigor, because inbreeding eliminates hybridity and allows weak recessives to express themselves.

12. In a state of nature, only the most vigorous pure lines survive. Species which inbreed (such as peas) are therefore vigorous. Inbreeding has no effect on their vigor, since they are pure.

13. Inbreeding brings weak recessives to the surface and hence assists natural selection in eliminating them from a population.

14. Heterozygosity prevents weak genes from expressing themselves and hence prevents natural selection from eliminating them. Outbreeding produces heterozygosity and hence tends to prevent the elimination of weak recessives by natural selection.

15. Outbreeding allows good mutations of separate origin to be brought together.

16. A combination of inbreeding and outbreeding leads to speedier evolution than either inbreeding or outbreeding by itself, since the one (inbreeding) assists natural selection in the elimination of weak recessives and the other (outbreeding) allows good mutations of separate origin to be brought together.

17. In bees, parthenogenesis assists natural selection, since it results in haploid offspring (males). In these, weak recessives express themselves and are eliminated by natural selection. The queens develop from fertilized eggs, and fertilization makes possible the bringing together of good genes

of separate origin. Hence bees combine the benefits of both inbreeding and outbreeding.

18. There is more inbreeding in small communities than in large, and hence weak recessives (as idiocy) have a better chance to express themselves in small communities. But the same thing applies to strong recessives, as those for talent.

PROBLEMS

1. Suppose we began with a large number of pink four-o'clocks $(w/+)$ and self-fertilized them for ten generations. What fraction of the offspring (in the eleventh generation) would still be pink?

2. How many pure lines might be produced by bean plants of genotype $\frac{a}{A} \frac{b}{B} \frac{c}{C} \frac{d}{D}$?

3. Suppose we began with a stock of, say, 800 mice of genotypes A/A and a/A in equal numbers and that we reproduced the stock by brother-sister matings in each generation. If the matings were random, what possible combinations of genotypes would occur among the parents? Would a family which happened to become homozygous ever produce anything but homozygous descendants (for all future generations)? Would the heterozygotes continually produce some homozygotes? Hence, what effect would brother-sister matings have on the genetic constitution of the stock of mice? What genotypes would the stock come to consist of eventually?

4. Given a family in which the offspring were of genotypes A/A and a/A. Tell whether or not a homozygote could give rise to anything but homozygotes (a) if the sexes were combined and only self-fertilization occurred, (b) if the sexes were separate and the offspring were inbred (brother-sister matings). Which form of inbreeding would therefore lead to the speedier attainment of homozygosity, self-fertilization or brother-sister matings?

5. In corn the tassels (or pollen-bearing organs) are separate from the pistils (the seed-producing organs) and are exposed to the wind. Which form of fertilization (self or cross) does this arrangement of the reproductive organs favor? If corn were naturally self-fertilizing, would it be dependent on hybridity for vigor? Tell why or why not.

6. In general if you were told that self-fertilization resulted in a reduction in vigor in one species of plants (A) but not in another (B), what conclusion could you draw as regards the form of breeding natural to the two species?

7. If we crossed two inbred lines of corn $(A \times B)$, the number of seeds produced by the cross would be small because inbred races of corn are not very fertile, but the seeds themselves would be vigorous and grow to be

very fertile plants. Like considerations apply upon crossing inbred lines C and D. Suppose you were the director of an agricultural station and had to supply farmers with large quantities of hybrid corn seeds (for growing crops of corn plants having hybrid vigor). How would you go about producing the seeds?

8. Assume that genes which are very close together in a given chromosome are transmitted in a group to the offspring, thus acting as though they were a single gene. Assume further that aB are close together in one chromosome and Ab in the homologous chromosome in a given plant $\left(\dfrac{aB}{Ab}\right)$, and that the plant is self-fertilized. Tell what classes of offspring this plant would produce. Would there be any offspring pure for both dominant genes $\left(\dfrac{A\ B}{A\ B}\right)$?

8. *THE GENETICAL INTERPRETATION OF SEX*

THE UNION of the sexes and the production of offspring are so intimately associated in man and in all the more familiar animals and plants that it would seem inherently impossible to have offspring without sexual union first. If the question is asked "Why are there male and female?", the obvious answer might seem, "In order that there might be offspring."

Asexual Reproduction.—Yet reproduction is not necessarily bound up with sex. In the bacteria reproduction simply involves the constriction of the parent into two in the process of cell division. Even in the higher organisms reproduction may take place directly, without the intervention of sex. A geranium plant can be reproduced by means of twigs or slips. The potato plant, as is well known, is ordinarily reproduced by cutting the "eyes" out of a potato and planting them. The banana lacks fertile seeds and can be reproduced only by means of slips; so too with the seedless orange. Among animals there are certain species of worms that chop themselves into segments and then grow into new worms from the segments. Some of the simpler animals such as hydra can reproduce by constricting off buds. All these are examples of *asexual reproduction;* none of them involves fertilization. We might in fact define asexual reproduction as *the production of offspring without fertilization.*

The Evolution of Sex.—The asexual method of reproduction, being simpler than the sexual method, probably came first in the course of evolution. This conclusion is confirmed by the fact that it is the exclusive way in which bacteria reproduce (so far as known), and these are the simplest organisms in the living world, apart from certain other organisms (viruses) which are too small to be visible under the microscope and which perhaps also reproduce asexually.

But even after sex made its appearance in the course of evolution, it probably did not do so in full-fledged form from the very start. Like everything else of a complicated nature, it must have developed gradually in the course of a long evolutionary process. Studies of primitive present-day organisms indicate that in the early stages of evolution fertilization involved merely the union of two cells that were essentially alike. At this stage, there was no differentiation into male and female. The parents were similar to each other; so were their reproductive cells. But gradually the reproductive cells of one parent became larger than those of the other and were loaded with food for the young. With their increase in size went a loss in their power of movement. They became the eggs. The cells of the other parent became smaller and highly motile. They evolved into the sperm cells.

Male and female were at first alike apart from the difference in their reproductive cells. It was only later that the two sexes came to differ in bodily form and activity. But even then the female at first simply shed her eggs into the water and left the young to shift for themselves as in many fishes and in frogs and other amphibia. Later, in some groups (birds) the female nourished and protected the young after she laid them. Finally (in man and other mammals), the mother retained the young within her uterus over a long developmental period and she continued to nourish and protect them after birth and during infancy. The female was handicapped during the time of caring for the young and became partially dependent upon the male for food and protection of the young, especially in species which had to get their food by hunting for it. It so happened, therefore, that a more or less permanent union was formed between the sexes, and the emotional relationship between male and female in man thus represents the culminating stage in the evolution of sex.

The Genetic Advantage of Sexual Reproduction as Compared to Asexual.—But let us now consider the biological advantage of sex. In species that reproduce only asexually, the offspring have just one parent from which they can inherit their genes. It is therefore not possible for a mutation to be introduced into one line from some other one. Sexual reproduction, on the other hand, makes possible outbreeding and allows two strains to come together each of which has good mutations that the other lacks. But the combination of two germ plasms would not in

itself bring about the concentration of good mutations in a given strain. For if the two germ plasms again separated at the reduction division without Mendelian recombination, then the mutations would again separate. Thus, if one strain contains the genes $a\ B$ and the other $A\ b$ (a and b being mutant genes), then by the sexual union of the two strains offspring of genotype $\frac{a\ B}{A\ b}$ might be produced. If there were no Mendelian recombination, the hybrid $\frac{a\ B}{A\ b}$ would produce only two classes of gametes—$a\ B$ and $A\ b$ —and it could therefore not produce any offspring pure for both mutant genes $\left(\frac{a\ b}{a\ b}\right)$. But if there were Mendelian recombination, then the hybrid $\frac{a\ B}{A\ b}$ would form some gametes of class $a\ b$ and by the combination of two such gametes, offspring might be produced of genotype $\frac{a\ b}{a\ b}$, pure for both mutant genes. Thus it is only through Mendelian recombination that the mutations in the two germ plasms can be concentrated into one. Hence the main advantage of sexual reproduction as compared to asexual is that it leads to *Mendelian recombination*. It was because of this advantage that sex became almost universal in the plant and animal kingdoms.

Reproduction in Paramecium.—There is an older interpretation of fertilization known as the "rejuvenescence" theory, based largely on observations made on a certain one-celled animal known as Paramecium (Fig. 53a). But first a word about the reproduction of Paramecium. From a single original parent large numbers of Paramecia can eventually be derived by simple cell division—a process of asexual reproduction. But under natural conditions Paramecia do not reproduce indefinitely by the asexual method. From time to time the Paramecia come together in two's—they *conjugate*. After remaining together for a while, they separate. They are then referred to as *ex-coniugants*. These and their descendents now multiply again by ordinary cell division (that is, asexually) until the next conjugation, as a rule.

Certain nuclear changes take place in two Paramecia during conjugation, changes that are equivalent to fertilization. In Paramecium the nucleus consists of a larger body or mega-nucleus and

a smaller body or micro-nucleus (Fig. 53a). During conjugation the mega-nucleus degenerates and the micro-nucleus undergoes

a. A SINGLE PARAMECIUM

b. TWO PARAMECIA CONJUGATING
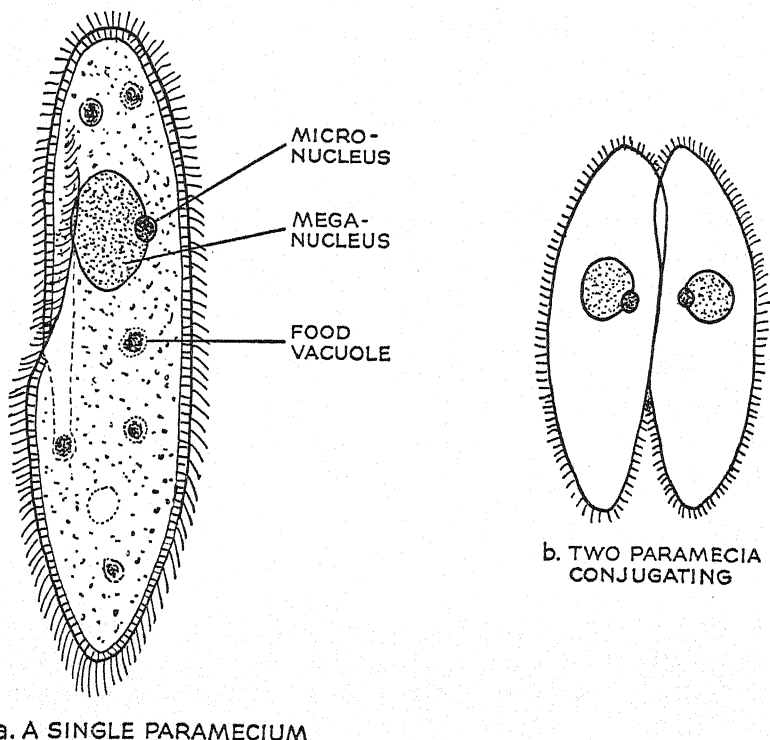
FIG. 53. Paramecium.

division, giving rise eventually to two daughter nuclei, one larger than the other (Fig. 54). These are not to be confused with the original mega- and micro-nucleus for they are sexual in nature.
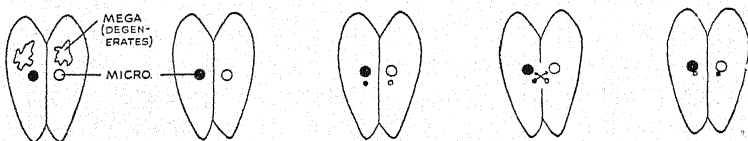
FIG. 54. Fertilization in Paramecium.

The larger of the two is the equivalent of an egg and remains in the Paramecium that produced it. The smaller nucleus is the equiva-

lent of a sperm cell. It migrates into the other Paramecium and there unites with the larger stationary nucleus (the egg). The fusion of the nuclei amounts to fertilization. From the fertilized egg nucleus new mega- and micro-nuclei are formed.

Paramecia have been kept for many years (by Woodruff) without conjugation; but in these lines there occurs another process, called *endomixis* by Woodruff. It has been shown by Diller that endomixis is really a case of self-fertilization or *autogamy*. During endomixis, the mega-nucleus degenerates. The micro-nucleus undergoes division and gives rise to a sperm and an egg nucleus. These nuclear changes are similar to those observed in conjugation. But in endomixis only one Paramecium is involved and the sperm nucleus of this Paramecium combines with its own egg nucleus.

The Rejuvenescence Theory.—If Paramecia are prevented from conjugating (by keeping them separate), they gradually die, as a rule. Some older biologists concluded from this that it was in the nature of protoplasm to die of old age, and that the function of fertilization was to prevent death from old age by "rejuvenating" the protoplasm. This interpretation of fertilization is known as the "rejuvenescence" theory. If this theory were true, then we should expect the bacteria to have passed out of existence a long time ago, since they reproduce only asexually, so far as is known. Moreover, potatoes and seedless oranges have been propagated over long periods of time uninterruptedly by asexual methods and are as vigorous as ever. Then again the giant sequoia trees of California have been in existence for over 2,000 years, yet their protoplasm shows no signs of senescence. Apparently it is not in the nature of protoplasm to lose its vitality through old age unless fertilization intervenes.

The Mating Behavior of Paramecium.—It might, however, be asked what causes conjugation in Paramecium. Before attempting to answer this question, a word might be said about the nature of sex in Paramecium.

Paramecium is really an hermaphrodite insofar as a given individual produces both male and female gametes in the form of a small migrant nucleus and a larger stationary one at the time of conjugation. But there is another kind of sexual differentiation in Paramecium. The work of Sonneborn, Jennings, and others has shown that within a given species of Paramecium the various

individuals differ in their mating responses with reference to one another in that some Paramecia will mate with each other, others will not. This is true even though the Paramecia are sexually mature. In Paramecium bursaria members of the species can be classified into three groups (I, II, III), such that none of the members of one group mate with the members of any other group. A given group in turn is divided into *mating types* such that none of the members of a given type mate with one another, but they do mate with any other type within their group. For example, Group I is divided into four mating types labeled A, B, C, D. The members of type A do not mate with one another, but they do mate with members of types B, C, and D. Likewise, one B does not mate with another B, but it does mate with A, C, or D. And so on.

Mating types are also found in *Euplotes patella,* a close relative of Paramecium. In Euplotes, Kimball has found that the mating types are determined by three genes (mt^1, mt^2, mt^3). All three belong to the same locus (they are alleles). A given animal might be pure for any one allele (as mt^1/mt^1), or it might be heterozygous for any two (as mt^1/mt^2). In all there are six possible combinations of the three alleles $\left(\dfrac{mt^1}{mt^1}, \dfrac{mt^1}{mt^2}, \dfrac{mt^1}{mt^3}; \dfrac{mt^2}{mt^2}, \dfrac{mt^2}{mt^3}; \dfrac{mt^3}{mt^3}\right)$ and thus there are six mating types. Each allele causes the production of a specific chemical substance, for each mating type produces a characteristic chemical substance in the water in which it swims. Animal-free fluid from a culture of one mating type induces conjugation among animals of certain other mating types if it is mixed with them. Fluid from one of the homozygous types induces conjugation only in animals which have no allele in common with it. Fluid from one of the heterozygous types induces conjugation among any other type than its own.

The Life Cycle of Paramecium.—After Paramecia have conjugated they are the equivalent of fertilized eggs. We can refer to them as *ex-conjugants.* All of the Paramecia derived by mitotic cell division from a single ex-conjugant are sometimes referred to as a *clone.* They are in a sense the equivalent of the body of a higher animal, since they are all derived from a single fertilized egg. A young clone corresponds to a developing embryo. Then follows the period of maturity, and now the Paramecia are capable of mating. The mating reaction involves among other things a

slowing of the movements of the Paramecia, as obviously is necessary if the Paramecia are to conjugate. It also involves an agglutination reaction in which the Paramecia become sticky and remain together upon contact. The mating reaction further involves a suppression of ordinary cell division. Finally, it involves nuclear changes leading to the formation of the sperm and egg nuclei, and to their union.

The depression period is not caused by old age; it is part of the mating reaction. It can be induced in very young lines of Paramecium (produced within a week or two after the last conjugation) by simply mixing together two mating types.

The Relation of Genetics to the Physiology of Fertilization.—Early in evolution the sex cells were probably very much like ordinary cells and could in the absence of fertilization reproduce asexually (by cell division). But, in the course of evolution, it became increasingly difficult for the sex cells to develop without combining. The species was, so to speak, forced to reproduce sexually. The change to forced fertilization must have been favored by natural selection. For it was of advantage to the species that the sex cells should not develop without first combining, because of the genetic advantage of sexual reproduction. Hence, those mutations which led to forced fertilization were selected. Undoubtedly these mutations produced physiological changes whereby some block (or check) was placed on the direct development of the egg. At the same time other mutations made it possible for the sperm cell to initiate development.

We may then consider fertilization from two points of view, one the genetical, the other the purely physiological. The geneticist can tell us of what survival value the sexual mode of reproduction is to the species, and why it replaced the asexual mode in the course of evolution. The physiologist on the other hand has still to discover many of the physical and chemical changes that accompany fertilization. It seems reasonable to assume that the genetical advantages of sexual reproduction led indirectly (through mutations and natural selection) to the physiological processes attendant upon fertilization. In brief, *the physiology of fertilization was dependent in the course of evolution on the genetical advantages of fertilization.*

SUMMARY

1. By *asexual reproduction* is meant the production of offspring without the intervention of fertilization.

2. Studies of the bacteria and other primitive organisms indicate that asexual reproduction preceded sexual in the course of evolution, and that sex itself underwent a gradual increase in complexity, beginning with the simple fusion of two cells in fertilization and ending with the differentiation of the sexes as seen in the higher forms of life.

3. Sexual reproduction permits of the concentration of good mutations into a single line through Mendelian recombination. It was because of this genetic advantage that sexual reproduction came to be almost universal in the plant and animal kingdoms.

4. According to an older theory (the "rejuvenescence theory"), all protoplasm tends to age, and fertilization is necessary for renewing its youth. Long-continued asexual reproduction is evidence against this theory.

5. Paramecia fall into mating types, each consisting of individuals which do not mate with one another, but which do mate with members of other mating types within their "group."

6. The evolution of sex involved physiological changes which insured fertilization. The mutations which led to these changes were selected because of the genetic advantage of sexual reproduction (Mendelian recombination). Thus the physiology of fertilization was dependent, in the course of evolution, upon the genetical advantage of sexual reproduction.

PROBLEMS

1. Given a plant of genotype $\dfrac{a}{A}\dfrac{b}{B}$. If this plant is reproduced asexually (by means of grafts or cuttings), how will all the progeny compare with one another and with the parent plant genetically? Why?

2. If a plant of genotype $\dfrac{a}{A}\dfrac{b}{B}$ is reproduced sexually (by means of seeds), will the progeny be genetically uniform? Why or why not?

3. If a plant were of genotype $\dfrac{A}{A}\dfrac{B}{B}$, how would the progeny compare as regards genetic uniformity if they were reproduced (a) asexually (b) sexually? Would you say, then, that sexual reproduction in itself led to increased genetic variation among the offspring? When there is increased genetic variation, what is the cause of it?

4. Apple and other fruit trees are usually reproduced by means of grafts, and the progeny are then very uniform and true to type. When, on

the other hand, they are grown from seeds, they lack uniformity. Why should they be uniform in the first instance but not in the second?

5. It is possible to take a small piece of a cancer from one rat and transplant it below the skin of another rat, where it then grows to full size (by mitotic cell division); and by a repetition of this process it is possible to grow millions of cancers from one original cancer and to continue the "line" over a period of many years, yet without any abatement in the "malignancy" of the cancer. What bearing have these facts on the rejuvenescence theory?

6. Would you say that parthenogenesis was evidence for or against the rejuvenescence theory? Why?

7. Among herbivorous animals (as cow, sheep, horse), would a given female be dependent on a given male for assistance in feeding herself and her young or for other assistance in the rearing of the young? Would you then expect the herbivores as a rule to be monogamous or polygamous?

9. *SELECTION*

SUPPOSE a group of people should migrate to an island with the object in view of producing a very tall race of human beings by breeding methods, very much like those employed by practical breeders of animals and plants. To what extent could they succeed? Let us assume that the group who start upon the venture are themselves of average size; further that they and their offspring continue the experiment over a large number of generations; and that in each generation only such persons as are taller than the average of the previous generation are selected as the parents for the next generation, all others moving off the island. Would a race of tremendous giants ultimately come into existence?

It would, in fact, be found that the race did increase in size and that the increase was comparatively rapid during the first few generations of selection. But the rate of increase per generation would gradually die down and during the later generations there would be no further change. That is to say, selection would be effective during the earlier generations of selection but not during the later generations.

The Limits of Selection in a Hybrid Population.—The results just described are somewhat comparable to what would happen to the skin color of a population if it began with F_1 mulattoes $\left(\dfrac{a}{A}\dfrac{b}{B}\right)$ and if selection for lighter skin color then took place. By the process of Mendelian reassortment the mulattoes would produce children of various genetic classes, most of whom contained some black genes. But there would be some who contained only the white genes $\left(\dfrac{a}{a}\dfrac{b}{b}\right)$. If they reproduced the next generation, then the average skin color of the population would be changed.

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But now the entire population would consist of persons who had nothing but white genes and further selection would then be ineffective unless mutations happened to occur that made the skin color still lighter. But mutations are very rare and differences in skin color would seldom be due to them. We therefore can ignore mutations for the purposes of our present discussion.

The effect of selection might extend over several generations instead of just one, but in this case it would take place through several small steps instead of just one large step. Thus the mulattoes with two white genes $\left(\dfrac{a\ b}{A\ B}\right)$ might have children with three white genes $\left(\dfrac{a\ b}{a\ B}\ \text{or}\ \dfrac{a\ b}{A\ b}\right)$ and these in turn might have children with all four white genes $\left(\dfrac{a\ b}{a\ b}\right)$. This increase is made possible through the process of Mendelian reassortment of genes. But by the same process the mulattoes with the two white genes can produce directly some offspring with all four white genes; and so the full effect of selection can be arrived at either in one large step or two smaller.

The important point is that no *new* skin color genes could come into existence through the agency of selection. The "white" genes are present at the start in the mulattoes. By Mendelian recombination they become assorted into the various classes of offspring, some of whom have more of them than others. Selection can *isolate* these classes; it cannot *produce* them. After the extreme type has been isolated there can be no further effect of selection in changing the average amount of pigment in the population (mutations excluded).

The results of selection for size would conform to a similar explanation. Size has a complicated hereditary basis. People of average size are usually hybrids and have an assortment of both tall and small size genes. Their offspring fall into various genetic types. Some have more genes for tallness than others, and by selection of the extreme type it is possible to change the average size of the population. After a race has been produced that is homozygous for all the genes for tallness, then further selection for size is ineffective (mutations excluded). This then is the *limit* of selection.

The Ineffectiveness of Selection Within a Pure Line.—But let us assume that we have got the human race homozygous for all its tall genes. Some people would still be taller than others. Would they not tend to have taller offspring? It would be difficult to perform an experiment on man in answer to this question because we could never be sure that the human stock was homozygous, and it is absolutely essential that we start with homozygous material; otherwise any effect of selection might be due to hybridity. We must therefore use experimental material which we are sure is not hybrid. For the purpose at hand beans are very favorable material because beans self-fertilize and self-fertilization eliminates hybridity, as we previously saw in connection with inbreeding. All the bean seeds on a given bean plant belong to the same pure line. Nevertheless, some are larger than others, possibly because they are in the middle of a pod instead of the end or because they grew on a well-sunned branch.

Suppose now we picked out one of the larger beans on the plant and grew it, would it produce seeds of larger size on the average than those of the plant from which it came? The answer is no. This was shown by the experiments of W. Johannsen of the University of Copenhagen. For example, Johannsen did one of his experiments on a pure line (Number VI in Table 3), the seeds of which had an average weight of 50.6 cg. (Table 3, second column), based on the weight of 141 seeds selected at random from a given bean plant (third column). He selected several seeds from this pure line, one of which weighed 30 cg. (second column under "Weight of Mother Bean"). He then got the offspring beans from this 30 cg. mother bean, and he found that their average weight as based on the measurement of 20 beans selected at random was 53.5 cg. (shown in the column headed "30" in Table 3). The offspring beans, in other words, weighed about the same on the average as the beans on the mother plant (a trifle more in fact—53.5 versus 50.6), despite the fact that the mother bean weighed 20.6 cg. less than the average of her line. A second mother bean selected from pure line VI weighed 40 cg.; the average weight of its offspring seeds was 50.8 (based on the measurement of 111 seeds selected at random). Here again the mother bean, though weighing less than the average of its line, produced offspring beans having approximately the same average weight as that of the pure line. In all, Johannsen worked with 19 pure lines (I–XIX), and the

results of all experiments are given in Table 3. It will be seen that all these experiments are consistent in showing that selection within a pure line is without effect.

TABLE 3. THE INEFFECTIVENESS OF SELECTION WITHIN PURE LINES (JOHANNSEN)

Pure Line	Avg. Weight (Centigrams)	No. Beans Weighed	Weight of Mother Bean					
			20	30	40	50	60	70
			Average Weight of Offspring Beans (Number of beans weighed shown in parentheses)					
I	64.2	(145)	....	....	....	....	63.1 (54)	64.9 (91)
II	55.8	(475)	....	....	57.2 (86)	54.9 (195)	56.5 (120)	55.5 (74)
III	55.4	(282)	....	....	....	56.4 (144)	56.6 (40)	54.4 (98)
IV	54.8	(307)	....	....	....	54.2 (32)	53.6 (163)	56.6 (112)
V	51.2	(255)	....	....	52.8 (107)	49.2 (29)	....	50.2 (119)
VI	50.6	(141)	....	53.5 (20)	50.8 (111)	....	42.5 (10)	....
VII	49.2	(305)	45.9 (16)	....	49.5 (262)	....	48.2 (27)	....
VIII	48.9	(159)	....	49.0 (20)	49.1 (119)	47.5 (20)	....	....
IX	48.2	(241)	....	48.5 (117)	....	47.9 (124)	....	....
X	46.5	(533)	....	42.1 (28)	46.7 (412)	46.9 (93)	....	....
XI	45.5	(418)	....	45.2 (114)	45.4 (217)	46.2 (87)	....	....
XII	45.5	(83)	49.6 (14)	....	....	45.1 (42)	44.0 (27)	....
XIII	45.4	(712)	....	47.5 (93)	45.0 (219)	45.1 (205)	45.8 (95)	....
XIV	45.3	(106)	....	45.4 (21)	46.9 (51)	....	42.8 (34)	....
XV	45.0	(188)	46.9 (18)	....	....	44.6 (131)	45.0 (39)	....
XVI	44.6	(273)	....	45.9 (147)	44.1 (90)	41.0 (36)	....	....
XVII	42.8	(295)	44.0 (78)	....	42.4 (217)	....	....	....
XVIII	40.8	(357)	41.0 (54)	40.7 (203)	40.8 (100)	....	....	....
XIX	35.1	(219)	....	35.8 (72)	34.8 (147)	....	....	....

Johannsen carried his experiments further. He continued to select the larger seeds for several generations. But he found that the average seed size was no greater at the end of selection than at the start, *provided* he began with a single bean plant. In beginning with a single plant, he made certain that he was confining himself to a single pure line in a given experiment. In summary, then, *selection within a pure line is ineffective.*

Selection Within a Mixture of Pure Lines.—If we ourselves wanted to perform a selection experiment on beans we might begin by going to the grocer's for a quart of beans, and after picking out one of the larger beans, plant it, wait for it to grow, and then gather the seeds that it produced. If now we measured these seeds for size we should probably find that their average

size was greater than that of the quart with which we began and we might think at first that our result was not in agreement with the one above described. But if we carried the experiment through another generation and planted one of the larger seeds of the first generation plant, we should discover that it produced seeds no larger on the average than those of the parent plant. In other words, we should get an effect of selection in the first generation but not thereafter. The reason is that the original quart contains a mixture of beans belonging to various pure lines, each of different average seed size. By selecting one of the larger beans from the quart, we isolated a pure line of larger seed size than the average and got an effect of selection, but after we had isolated the pure line we got no further effect of selection.

Overlapping Phenotypic Variation in a Mixture of Pure Lines.—There is no way of telling from mere inspection that a quart of beans is a mixture of different pure lines, each having its own average size. If, indeed, there were just two lines in the mixture, one very large and one very small with no intermediates, it would be perfectly apparent that there were two distinct kinds of beans. But there are lines of intermediate sizes connecting the two extremes, and we get the impression that we have just one kind of bean which varies in size.

Not only are there pure lines of intermediate size connecting the two extremes but in addition the beans within each pure line vary in size, and the largest beans of one line *exceed* in size the smaller of the next higher class. In other words, there is an *overlapping* of the range of variation in the size of the two lines. It is therefore impossible to decide by mere inspection whether a particular bean within the range of the overlap belongs to one pure line or the other. To decide this point we should actually have to plant the bean and find out the average size of its offspring. These would not necessarily conform to the particular size of the parent seed but to the average of the pure line to which they belonged. The parent seed departed from the average of its class not because it was different in its hereditary make-up from the others of its class, but because of the influence imparted by the particular environmental conditions under which it developed, an influence which cannot be transmitted to its offspring.

We can refer to the entire range of variation in the appearance of a given pure line (as in its size), as its phenotypic variation.

In summary then, within a mixture of pure lines there is overlapping *phenotypic* variation, but no overlapping *genotypic* variation.

The Stability of the Gene.—If the genes in a bean plant underwent continual variation from one generation to the next, then selection within a pure line would be effective. But Johannsen's experiments definitely show that selection within a pure line is without effect. This proves that genes do not undergo continual variation. Further experiments have been conducted with the object of testing the stability of the gene and whenever they have been properly controlled, they have confirmed for other forms of life the results found to apply to beans. Some genes are more mutable than others, but even the most mutable are comparatively stable, and the vast majority of genes are exceedingly stable, though they do mutate occasionally.

The Practical Results of Selection.—Despite the limitations that there are on selection, practical breeders have accomplished truly amazing feats by means of it. Cows that produce over half a ton of butterfat a year, hens that lay an egg a day, sweet peas and roses of exceeding beauty and bewildering variety—these and many other results they have obtained through the magic of selection. Breeders seem, moreover, to be able to get almost anything they want; tremendous dogs for hunting big game or little ones for ladies to hold in their laps; horses that do a half mile in less than a minute or that move a heavy load almost through sheer bulk. They seem to hold the race in their hands and to get a product very different from the thing with which they begin. To be sure, they are very patient. Their work extends over many years and one may only begin a thing that many others help to carry to completion in later generations.

The history of the practical breeder's work is often obscure. Some things came from China many hundreds of years ago—tea, the silkworm, and the primrose, among others. Corn was under cultivation and in a high state of development at the hands of the American Indians before ever the white men arrived in the new world. The hen, some think, came originally from India, but whether from one original wild race or through the crossing of two is not definitely known. The cat was known to the ancient Egyptians. Primitive man already had the dog as a companion. Possibly he got the dog by domesticating the wolf or the jackal (a kind of wild dog that closely resembles the domesticated dog).

Even after the various domesticated races of animals and plants came into the possession of Western Europeans, there was often no definite record made of exactly what they were like at the time, nor of the further changes that they underwent. The breeder of domesticated animals and plants was and still is a practical man, not a theoretical scientist. He is more interested in getting his results than in how he gets them. In consequence he has often left us no definite records. But we know in a general way how the breeder got his results. Sometimes he crossed different races and produced hybrids from which he got new types by selection in later generations. The new types were produced by Mendelian recombination. When a race was already more or less hybrid it was not necessary first to cross it to another race to get results. Selection by itself sufficed. Or, still more simply, the breeder sometimes selected something already existing in nature and merely requiring favorable cultural conditions to make it suitable to human needs. Burbank often got results in this way.

In very rare instances something genuinely new suddenly arose not attributable to hybridization. A case in question was the very short legs of a certain breed of sheep, the Ancon breed, so short that they saved farmers the trouble of building high fences around enclosures from which ordinary sheep could escape by jumping. The new breed of sheep appeared unexpectedly and independently of any attempts at producing it through selection; it was merely seized upon and saved after it made its appearance. Changes of this sort are mutations. They have arisen from time to time in domesticated races of animals and plants and have been of value to the breeder. This is especially true in the case of races that have been kept under observation for centuries, in the course of which the mutation process has been given sufficient time to produce many valuable changes. Some of these changes were doubtless brought about by large steps, but others by a series of small steps. Mutations in fact are more often small than large. The improvement in cows and horses has been due mostly to the slow accumulation of many small mutations. Not in all instances have mutations been valuable. Sometimes they deformed a plant or animal and caused it to be cast aside as a freak or monstrosity.

The Limitations of the Breeder's Methods.—Whatever the methods of the practical breeder may have been, one thing is

fairly certain: the breeder cannot by mere selection *produce* new genes. When, for example, the breeder produces a tall race from one of the average size he does not create the genes for tallness. What he does for the most part is merely to isolate a Mendelian class. Once he gets the extreme type made possible by the genes already in existence, he can get no further improvement by the selection of Mendelian classes. The only possibility that then remains for further progress is mutation. This the breeder cannot control, in the sense of causing a mutation of the desired type. Selection for tallness would not in itself cause mutations for tallness.

The Effectiveness of Selection in Small Steps.—It might seem that if selection from hybrid stock is capable only of *isolating* Mendelian classes, then the breeder's task ought to be finished in one generation. For in one generation the hybrids can produce all possible Mendelian classes. Still, the breeder can effectively carry the process of selection beyond the first generation. What is more, selection itself seems to predispose the race towards changing further in the direction that selection got it started on. Thus once the breeder has got it on the way towards tallness, it seems to continue going, much like a ball that continues to roll from the impulse originally imparted to it.

An illustration will suffice, possibly, to make clear why this should appear to be so, as well as why selection can be carried effectively over several generations, beginning with hybrid stock. Take again for the sake of simplicity the mulatto and selection for lighter skin color from original mulatto stock. Mulatto parents, each having two white genes, can produce offspring of the extreme type having all four white genes, as well as offspring of the other extreme having no white genes at all (but four black in place of them). These extremes are rarer than the intermediates having one, two, and three white genes; and if the number of offspring were limited, only the intermediates would make their appearance as a rule. This point has already been mentioned, as well as the further point that from the offspring having three white genes, others having all four could be produced in the next generation, and that the effect of selection could therefore be carried over two generations. But note a further fact. Offspring with three white genes are more likely to produce some with four than were the original hybrid parents (who had only two), simply because it

is a smaller step from three to four than from two to four. The offspring tend to vary about the average of their immediate parents in the number of white genes they receive and so each generation would naturally enough vary in the direction of selection, until the extreme Mendelian class was secured.

But the extreme class in question would actually be got with *fewer* numbers if selection is allowed to take place over several generations than if it is forced to get the desired class in just one generation. For, in order to insure the production of an extreme class in one generation by Mendelian recombination, it would be necessary to raise enough offspring to get not only this class but also the other extreme as well as all the intermediates. If, on the other hand, selection takes place in several steps, the one extreme makes its appearance *without* the other and without the relatively large numbers of offspring that would be necessary to insure the production of both extremes and all the intermediates. It is possibly on this account that selection *seems* to predispose the race towards changing in the desired direction and to cause something to come into existence that would not otherwise have appeared. Furthermore, as the number of genes goes up that characterize the extreme classes, the number of offspring necessary to insure their appearance in a single generation goes up out of all proportion, and it becomes practically impossible in an ordinary experiment to get the required numbers of offspring when the number of gene pairs involved gets beyond a dozen or so. This difficulty is not encountered when the selection experiment is carried on in small steps over several generations.

Back and Forth Selection.—There is still another thing about selection that appears rather surprising. It is possible to change a race first in one direction by selection, then back again in the direction from which it came, and to do this back and forth at will. Now if it is true that the extreme type got by selection is merely an isolated Mendelian class, then this sort of thing would not be expected, especially if the class in question were uniform in its genetic constitution and of pure type. Further selection, whether back again or further along, should be ineffective, for the same reason that it is ineffective in a pure line. And in fact it would be, if the experimenter could be sure that he had actually isolated the extreme class and this class only. But in point of fact it is difficult to be sure of this. If some hybridity still exists or

if some individuals in the race are not actually of the extreme genetic class but only apparently so, then it is possible by selection to return the race to the original type. For if either of these things is true, it means that there are still in existence some genes of the old type, scattered about in the population; and it is possible by crossing and selection to concentrate these in the course of several generations into a few individuals and to get from them again a race of the original type.

The Production of F_2 Classes More Extreme Than Either Parent Race.—When hybridization is combined with selection, it is possible to get from two races extreme types which neither race by itself was capable of producing. For instance, hybrids can be produced by crossing two races of tobacco differing in the size of their flowers, and from the hybrids offspring can be got some of which are of larger flower size than that of the large race and others of smaller flower size than that of the small race, as well as intermediates. This is due to the fact that each parent race contains genes for size that the other lacks, and by crossing and Mendelian recombination it is possible to get all the large size genes together into some of the descendants and all of their allelic small size genes into others, and so to produce two types each more extreme in its direction than the original parent races. Thus if *ABC* are genes for large flowers and *abc* for small, then the larger race might have two of the large size genes and one small, say, *ABc* and the small race two small and one large, *abC*. The cross would be $\dfrac{ABc}{ABc}$ (large-flowered race) $\times$ $\dfrac{abC}{abC}$ (small-flowered race).

The F_1 would be $\dfrac{ABc}{abC}$. By inbreeding these we could get in the F_2 two extreme classes (1) $\dfrac{ABC}{ABC}$ and (2) $\dfrac{abc}{abc}$. Each class would be more extreme for flower size than either parent race $\left(\dfrac{ABc}{ABc}\right.$ and $\left.\dfrac{abC}{abC}\right)$.

When the extreme types already exist before hybridization as in the Negro-white cross, it is not possible through hybridization and selection to go any further in the direction of the extremes. But when each parent has genes for a given extreme that the

other lacks, then it is possible to pool their genes through hybridization and to lump together the genes of the extreme types by means of Mendelian segregation in the hybrids. In this way we can get F_2 classes more extreme than either parent class.

Two races might be alike in outward appearance, and yet by crossing and selection it might be possible to get extremes which neither race by itself is capable of producing. Thus suppose two races of man had become isolated on separate islands and were of intermediate skin color. It would be entirely possible for each race to be of pure type, from the genetical viewpoint, in spite of their outward resemblance to the mulatto, a hybrid type. To use symbols, one might be $\dfrac{A}{A}\dfrac{b}{b}$, the other $\dfrac{a}{a}\dfrac{B}{B}$. A cross of the two races would produce F_1 of genotype $\dfrac{A}{a}\dfrac{b}{B}$, and from the F_1 it would be possible to obtain offspring of the extreme types $\dfrac{A}{A}\dfrac{B}{B}$ and $\dfrac{a}{a}\dfrac{b}{b}$.

The Promise of Selection.—It will be apparent now that selection has its limitations. It cannot call new genes into existence; it can only operate on those that are already at hand, or on those that appear accidentally as mutations. It is well that we should know what selection cannot do, as well as what it can. For then we are spared the trouble of trying to make it do the impossible, and we use it for what it can do. It still has tremendous possibilities. In almost every race there exist the scattered possibilities for its improvement; and by means of selection, or hybridization and selection, the breeder can bring genes together into all conceivable combinations, suitable to the needs and fancy of man. Of this fact the workers at a certain experiment station in Sweden (Svalöf) have given a convincing demonstration. By a careful study of wheat, they were able to discover varieties already in existence that were suitable to the many different soils and climatic conditions of Sweden, or that furnished the material for crosses suitable to these ends.

In the case of the human race the possibilities for improvement are still more far-reaching, especially in the field of mentality. Here we find the possibilities of a Shakespeare, a Darwin, and a

Pasteur. Only after selection has brought the race to the level of these and possibly even greater will it have reached its goal. Then it will be time enough for the slow process of mutation to carry the race further. Who knows but that the process of mutation itself might not by then be well under control?

SUMMARY

1. In a hybrid population, selection for increased size would be effective at first but would no longer be effective after the population had become homozygous for all the genes for tallness, unless mutations happened to occur.

2. Selection within a pure line is without effect. This is shown by the fact that the heavier seeds on a bean plant produce offspring seeds which are no heavier on the average than those produced by the lighter seeds on the mother plant.

3. If a bean is selected from a mixture of pure lines, it produces offspring beans having the average weight of the pure line to which the mother bean belongs. If the mother bean belongs to one of the heavier lines, then it produces offspring seeds which are heavier on the average than those of the mixture. But further selection within the pure line thus isolated is without effect. In other words, selection isolates a pure line from a mixture of pure lines and is effective for one generation but not thereafter.

4. In a mixture of pure lines the heaviest beans of one pure line often exceed in weight the lightest beans of the line next above it in weight. Hence in the mixture it is often impossible to tell to what pure line a bean belongs by mere inspection of the bean.

5. The pure line experiments show that genes are very stable in the sense that they do not undergo continual variation from one generation to the next.

6. Selection has produced great improvements in our domesticated animals and plants.

7. Selection cannot produce new genes; it can only isolate Mendelian classes which contain desired genes.

8. Hybrids are capable of producing all possible Mendelian classes in one generation, but when the number of gene pairs is large, extremely large numbers of offspring are required for the recovery of the extreme classes in one generation. Hence it is often necessary to carry on selection over a large number of generations for the recovery of an extreme class of the desired type.

9. "Back and forth" selection is possible in a given stock so long as all members of the stock are not homozygous and of the same genotype.

PROBLEMS

1. Examine Table 3 (p. 170) and determine in how many pure lines a 30 cg. mother bean produced offspring beans (1) heavier (2) lighter, on the average, than did a 50 cg. mother bean of the same line (in those lines in which figures are given for both 30 and 50 cg. mother beans). Tell in how many lines a mother bean of 20 cg. produced offspring beans (1) above (2) below the average weight of the pure line from which they were derived.

2. Suppose *ABCD* were genes for heavy seeds and *abcd* for light seeds in beans, and that each "heavy" gene caused an equal increase in weight. Then how would the average weight of line $\dfrac{A\,B\,c\,d}{A\,B\,c\,d}$ and $\dfrac{a\,b\,C\,D}{a\,b\,C\,D}$ compare? How could you determine that these were two distinct pure lines?

3. In Table 3 (p. 170) the average weight of pure lines XI and XII is 45.5. How did Johannsen know that they were two distinct pure lines?

4. Suppose that you were given two bean seeds, each weighing 40 cg. but that one came from a line having an average of 55.8 and the other from a line with an average weight of 35.1 cg. What approximately should be the average weight of the offspring beans of each seed? Examine Table 3 to see if you have the correct answer.

5. In horses, cattle, and lower animals in which it is possible to mate one male to many females (or artificially to inseminate very large numbers of females with sperm from one male), do you think the improvement of a race could be more rapidly made through the selection of males or of females?

6. Assume that high milk production depends on a large number of multiple factors (*A*, *B*, *C*, *D*, etc.) and that bulls as well as cows might carry such genes. To what extent might a single bull raise the milk production of an entire herd of cows in one generation if the cows we began with had zero milk production and the bull were pure for all the genes for very high milk production (100 per cent)? In about how many generations could the production of the herd be raised to over 90 per cent by means of a single 100 per cent bull in each generation?

7. Given two bulls differing in the number of genes they carry for high milk production. Given also a herd of cows of average milk production. How could you determine which bull carried the larger number of genes for high milk production?

8. In attempting to improve the milk production of a herd of cows, would it be advisable merely to select the highest milk producers and breed them to any bull? What procedure would you suggest for improving the milk production of the herd?

10. *LINKAGE, CROSSING OVER, AND CHROMOSOME MAPS*

W HEN two genes are in the same chromosome, they are tied to one another and are said to be *linked*. They tend to be transmitted together to the offspring. But sometimes a pair of chromosomes exchange segments at the reduction division, as shown in Fig. 55. This exchange is known as *crossing over*.

CROSSING OVER

THE REDUC-
TION DIVISION
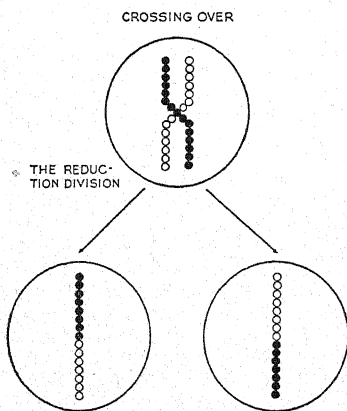
Fig. 55. Crossing over.

Suppose now that an egg cell contained a chromosome with the two genes A and B and that a sperm cell contained a and b. The combination of the two gametes would produce a hybrid of genotype $\dfrac{a\ b}{A\ B}$. (The *unbroken* line in the formula $\dfrac{a\ b}{A\ B}$ indicates that the genes are in one pair of chromosomes.) When the hybrid $\dfrac{a\ b}{A\ B}$ forms its gametes, A and B as a rule separate as a unit from a and b, giving two classes of gametes, $A\ B$ and $a\ b$ (Fig. 56, upper left). These are the combinations as they originally entered the hybrid, or the *old combinations*. But sometimes there is crossing over, and this results in the *recombinations* $A\ b$ and $a\ B$ (Fig. 56, upper right). In all cases of linkage the old combinations ($A\ B$ and $a\ b$) exceed the new ($A\ b$ and $a\ B$), when the hybrid forms its gametes. In other words, the old combinations are over 50 per cent of the total gametes, the new less. By contrast, when gene

pairs are in separate chromosome pairs, there is independent assortment, and so the old and the new combinations are equal (Fig. 56, lower part). We might, then, define *linkage* as the

LINKAGE

THE REDUCTION DIVISION

OLD COMBINATIONS (AB AND ab)
MORE THAN 50% OF THE TOTAL

NEW COMBINATIONS (Ab AND aB)
LESS THAN 50% OF THE TOTAL

INDEPENDENT ASSORTMENT

THE REDUCTION DIVISION

OLD COMBINATIONS (AB AND ab)
50% OF THE TOTAL

NEW COMBINATIONS (Ab AND aB)
50% OF THE TOTAL
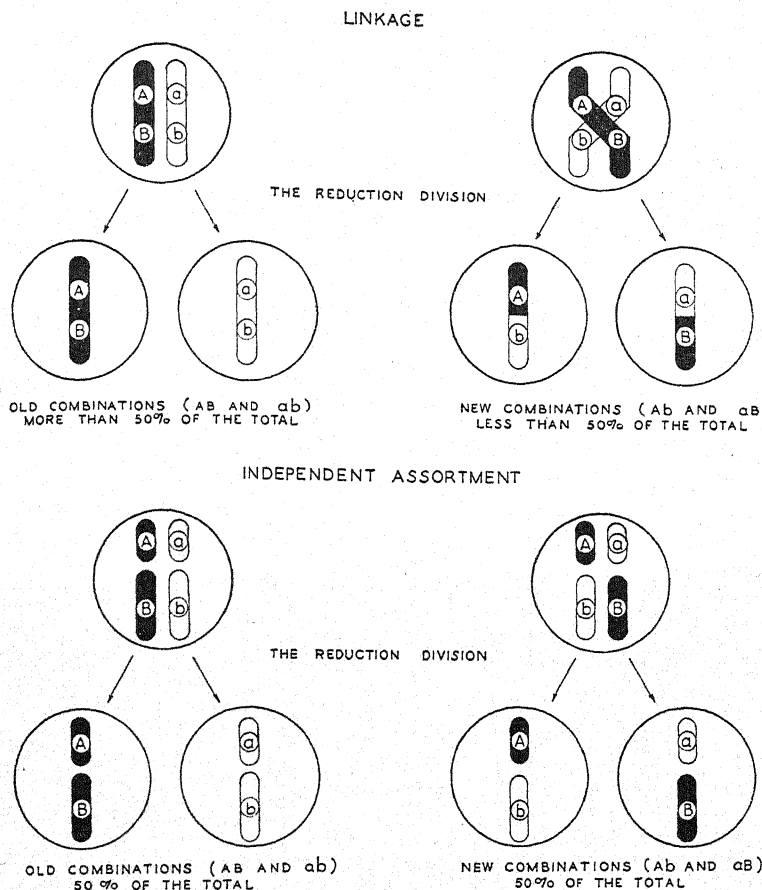
FIG. 56. Linkage and independent assortment compared.

tendency of two genes in the same chromosome to remain together in the process of inheritance. But crossing over sometimes separates genes which are linked.

The amount of crossing over between linked genes varies with their distance apart in the chromosome. The further apart they are, the greater the amount of crossing over between them. But

the new combinations of genes formed by crossing over never exceed the old combinations in amount; that is to say, they are always less than 50 per cent of the total. They may be anything from 1 per cent or less to almost 50 per cent of the total.
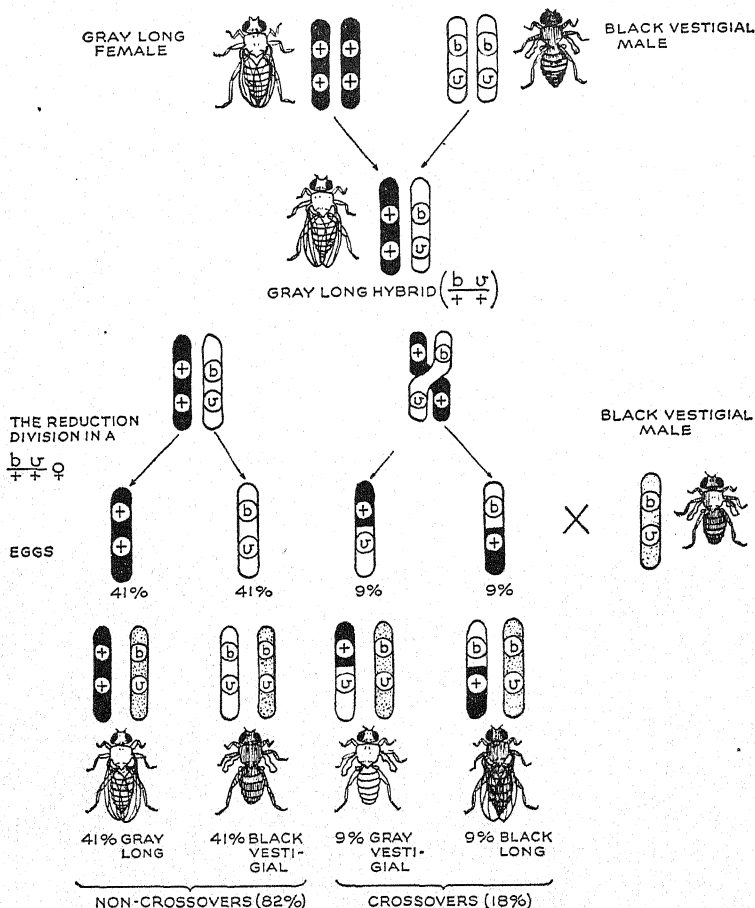
FIG. 57.1. A case of linkage in Drosophila (gray long × black vestigial).

A Case of Linkage.—To take a concrete case in illustration of linkage. In Drosophila the ordinary normal fly has gray body and long wings (Fig. 57.1). But a race has been got with the recessive mutations black body (*b*) and vestigial wings (*v*), both in the same chromosome. We can indicate the normal alleles of *b* and *v*

by $+$ signs. When a pure gray long is crossed to a black vestigial $\left(\dfrac{+\ +}{+\ +} \times \dfrac{b\ v}{b\ v}\right)$, the gametes of the two parents combine ($+\ +$ and $b\ v$), giving offspring of genotype $\dfrac{b\ v}{+\ +}$.

Figure 57.1, middle part, shows the reduction division in an F_1 female of genotype $\dfrac{b\ v}{+\ +}$. First, the chromosomes might come together and separate without crossing over between loci b and v, giving eggs of the two non-crossover classes $+\ +$ and $b\ v$. The second possibility is that crossing over might take place between loci b and v, giving eggs of the two crossover classes $+\ v$ and $b\ +$. The above four classes of eggs are formed in approximately the following proportions: $41\ +\ + : 41\ b\ v : 9\ +\ v : 9\ b\ +$.

We might now cross the F_1 female to a black vestigial male, thus $\dfrac{b\ v}{+\ +}\ \female \times \dfrac{b\ v}{b\ v}\ \male$. Since the male is pure for $b\ v$, he adds only $b\ v$ to each of the above four classes of eggs (Fig. 57.1, lower part). Accordingly, if we put the genes contributed by the eggs above a horizontal line and those contributed by the sperm cells below, the offspring of the cross are produced in approximately the ratio of $41\ \dfrac{+\ +}{b\ \ v}$ (gray long) : $41\ \dfrac{b\ v}{b\ v}$ (black vestigial) : $9\ \dfrac{+\ v}{b\ v}$ (gray vestigial) : $9\ \dfrac{b\ +}{b\ v}$ (black long.). The last two classes are the crossovers. They form 18 out of every 100 offspring, and therefore there is 18 per cent of crossing over between black and vestigial.

In the $F.$ cross just described $\left(\dfrac{b\ \ v}{+\ +}\ \female \times \dfrac{b\ v}{b\ v}\ \male\right)$ the offspring tell us directly in what proportions the F_1 female produces her eggs. This is due to the fact that the offspring receive only recessive genes from the male (namely, $b\ v$). It will be recalled that a cross of a hybrid by a recessive is known as a *test cross*. In linkage experiments the test cross is the simplest way in which to determine the per cent of crossing over.

A female hybrid for both b and v would not necessarily have to be of composition $\dfrac{b\ \ v}{+\ +}$ (with both mutant genes in one chromosome and both normal alleles in the other). She might equally

well be $\dfrac{b\,+}{+\,v}$, with $b\,+$ in one chromosome and $+\,v$ in the homologous chromosome. In such a female the non-crossovers would be $b\,+$ and $+\,v;$ the crossovers $b\,v$ and $+\,+$. Each non-crossover class would form approximately 41 per cent of the total gametes and each crossover class 9 per cent, just as when both mutant genes are in one chromosome and the two normal genes in the homologous chromosome. These facts could be shown by the test cross $\dfrac{b\,+}{+\,v}\ \female \ \times \dfrac{b\,v}{b\,v}\ \male$. The offspring now would be formed in approximately the ratio of 41 $\dfrac{b\,+}{b\ \ v}$ (black long): 41 $\dfrac{+\,v}{b\ \ v}$ (gray vestigial): 9 $\dfrac{+\,+}{b\ \ v}$ (gray long): 9 $\dfrac{b\,v}{b\ \ v}$ (black vestigial). The crossover classes form 18 per cent of the total offspring, and so there is 18 per cent of crossing over between black and vestigial in this cross, just as there was in the previous one.

The Use of Crossover Percentage as an Index of the Distance Between Two Genes in a Chromosome.—Presumably crossing over may occur anywhere along the length of a pair of chromosomes, at the reduction division, as shown in Fig. 57.2. The chances of crossing over are therefore greater over long distances than over short. Thus, in Fig. 57.2 there is more chance of crossing over between a and c than between a and b, since distance ac includes ab. Therefore, the per cent of crossing over between two genes depends on their distance apart. The farther they are apart, the greater is the per cent.

Assume now that the per cent of crossing over is always the *same* over any two segments of equal length (as ab and bc in Fig. 57.2). Then the per cent of crossing over between two genes is directly proportional to their distance apart. Thus in Fig. 57.2 there is twice as much crossing over between a and c as between either a and b or b and c. We may therefore use the per cent of crossing over between linked genes as an *index* of their distance apart. If there is 1 per cent of crossing over between two genes, we say that they are a distance of one "unit" apart; if there is 2 per cent of crossing over, the distance is two units; and so forth. But we do not know how long these units are in fractions of an inch. To give an analogy, if we knew that 10 drops of rain struck a clothesline between clothespins 1 and 2 in a given time and 20 struck the

line between 2 and 3 in the same time, we should know that 2 and 3 were about twice as far apart as 1 and 2, but we should not know in actual feet or inches how far apart 1, 2, and 3 were. We might, however, agree on calling the distance 1 to 2 one unit; then distance 2 to 3 would be two units. Just so with the distances between genes as determined by crossover per cents. The distances are *proportional* to the crossover per cents. However, this statement is true only on the assumption that the per cent of crossing over is the same, on the whole, over any two chromosome segments of
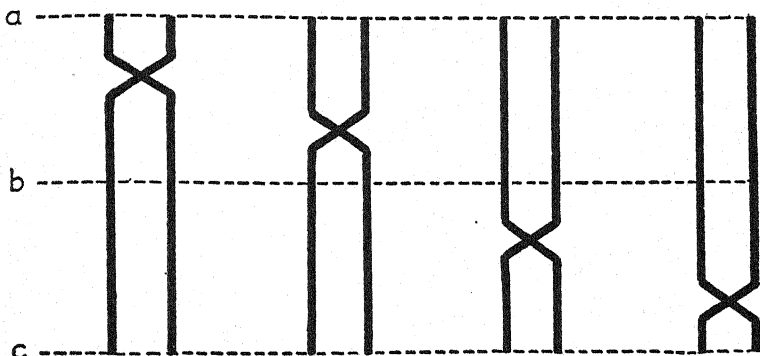
FIG. 57.2. Diagram showing equal amounts of crossing over, over equal distances.

equal length. This assumption seems reasonable, and in fact is supported by experimental evidence to be considered later.

The Absence of Crossing Over in the Male of Drosophila. —It so happens in Drosophila that there is no crossing over in the male. Thus if the male were of genotype $\dfrac{b \quad v}{+ \quad +}$, only two classes of sperm cells would be formed, $b\,v$ and $+\,+$. If the male were of genotype $\dfrac{b \quad +}{+ \quad v}$, the two classes of sperm cells would be $b\,+$ and $+\,v$. These facts could be determined in each instance by means of the test cross.

In organisms other than Drosophila there is not necessarily a lack of crossing over in the male. In rats crossing over has been found in the male, though it is somewhat less than in the female. There is, in general, a tendency for crossing over to be somewhat reduced in the heterozygous sex.

A Case of Linkage Involving Two Sex-linked Mutations.—
In Drosophila white eyes (w) and miniature (small) wings (m)
are two recessive mutations in the X chromosome. We can indicate their normal alleles by $+$ signs (for red eyes and long wings).
It will be recalled that in Drosophila a female has two X chromosomes and a male only one. A white miniature female would
therefore be $\dfrac{w\,m}{w\,m}$; a red long male is $\dfrac{+\,+}{\quad}$. (The blank space
below the line indicates the empty Y.) By crossing the white
miniature female and long red male $\left(\dfrac{w\,m}{w\,m}\ \female \times \dfrac{+\,+}{\quad}\ \male\right)$ we could
get F_1 females of genotype $\dfrac{w\,m}{+\,+}$. A $\dfrac{w\,m}{+\,+}\ \female$ forms four classes of
eggs: two non-crossover classes $(+\,+$ and $w\,m)$ and two crossover
classes $(+\,m$ and $w\,+)$, in the ratio of $34\,+\,+ : 34\,w\,m : 16\,+\,m : 16\,w\,+$.

Suppose now that the above hybrid female were mated to a
white miniature male $\left(\dfrac{w\,m}{+\,+}\ \female \times \dfrac{w\,m}{\quad}\ \male\right)$. Then the four classes
of egg cells might be fertilized either by the X-containing sperm
cells (with $w\,m$) or by the Y-containing sperm cells (without
$w\,m$). The X-containing sperm cells would add $w\,m$ to each of
the four classes of egg cells, giving offspring in the ratio of 34
$\dfrac{+\,+}{w\,m} : 34\,\dfrac{w\,m}{w\,m} : 16\,\dfrac{+\,m}{w\,m} : 16\,\dfrac{w\,+}{w\,m}$. These are all female offspring,
since they contain two X's. The sons on the other hand result
from the union of the "empty" Y-containing sperm cells with
the eggs and are formed in the ratio of $34\ \dfrac{+\,+}{\quad} : 34\ \dfrac{w\,m}{\quad} : 16$
$\dfrac{+\,m}{\quad} : 16\ \dfrac{w\,+}{\quad}$. Among both sexes, the crossover classes form 32
per cent of the total offspring (each crossover class forming 16 per
cent, the two together 32 per cent of the total). The distance
between w and m is therefore judged to be 32.

The cross just described $\left(\dfrac{w\,m}{+\,+}\ \female \times \dfrac{w\,m}{\quad}\ \male\right)$ is a test cross.
In describing this cross we first gave the ratio in which the F_1
female forms her eggs, and then we derived the offspring. But

actually we must first get the offspring and from these we derive the gametic ratio in the hybrid female.

Suppose next that the hybrid female had been mated to a red long male $\left(\dfrac{w \; m}{+ \, +} \; ♀ \times \dfrac{+ \, +}{} \; ♂ \right)$. Then the daughters would all receive $+ \, +$ from their father and all would appear normal. But the sons would receive the empty Y from their father, just as in the test cross, and so they would be formed in the same ratio as in the test cross $\left(34 \dfrac{+ \, +}{} : 34 \dfrac{w \; m}{} : 16 \dfrac{+ \; m}{} : 16 \dfrac{w \; +}{} \right)$.

Whenever a linkage experiment involves genes in the X chromosome, the sons of the F_1 female are in effect the product of a test cross, and they tell us how much crossing over there is in their hybrid mother, regardless of what their father might have been. This makes the X chromosome easier than other chromosomes to work with in linkage experiments.

Double Crossing Over.—Sometimes crossing over occurs at two points in the same chromosome pair as shown in Fig. 58. This is known as "double crossing over." The gametes formed by double crossing over are called double crossovers. By contrast crossing

FIG. 58. Double crossing over.

over just once is known as single crossing over and the resultant gametes are called single crossovers. The amount of double crossing over between two loci increases with the distance apart of the loci, but as a rule the doubles are fewer than the singles.

Crossing over might also occur at three points in the same chromosome pair (triple crossing over), but over short distances there are very few if any triples. Quadruples would be still fewer.

A Cross Involving Three Linked Genes in Drosophila (Yellow, Miniature, and Forked).—Let us take a specific case in illustration of double crossing over. In Drosophila yellow (body), miniature (wings), and forked (bristles) are three recessive mutations in the X chromosome (the normal fly having gray body, long wings, and straight bristles). We can indicate the mutant genes by the symbols y (yellow), m (miniature), and f (forked) and their normal alleles by $+$ signs. A yellow miniature forked female is $\dfrac{y \; m \; f}{y \; m \; f}$, a male is $\dfrac{y \; m \; f}{}$. A pure gray long straight female

is $\dfrac{+\;+\;+}{+\;+\;+}$, and a male $\dfrac{+\;+\;+}{}$. By crossing, say, a yellow minia-

ture forked female with a gray long straight male $\left(\dfrac{y\;m\,f}{y\;m\,f}\,♀\;\times\right.$

$\left.\dfrac{+\;+\;+}{}\,♂\right)$, we could get an F_1 female of genotype $\dfrac{y\;\;m\;\;f}{+\;+\;+}$

(Fig. 59).

When the reduction division takes place in the above female the chromosomes might pair in four possible ways, as shown in Fig. 59. Each kind of pairing results in two classes of eggs (known as contrary classes). The first and second classes represented in the figure are the non-crossovers ($+\;+\;+$ and $y\,m\,f$). The third and fourth classes ($+\;m\,f$ and $y\,+\,+$) are single crossovers between y and m, the fifth and sixth classes ($+\;+\,f$ and $y\,m\,+$) are single crossovers between m and f. The seventh and eighth classes ($+\;m\,+$ and $y\,+\,f$) are double crossovers between y and f. The approximate per cent which each class of eggs is of the total is given below the class in Fig. 59.

Suppose now that we crossed the hybrid female to a yellow miniature forked male $\left(\dfrac{y\;\;m\;\;f}{+\;+\;+}\,♀\;\times\dfrac{y\,m\,f}{}\,♂\right)$. Then we should

add the X of the male with the genes $y\,m\,f$ to each class of egg cells to get the female offspring, and the Y of the male with no genes (at the loci in question) to get the male offspring. Therefore the offspring, both male and female, are produced in the same ratio as that in which the eggs are produced in the F_1 female. In actual practice we should first get the offspring from the test cross and these would tell us in what percentages the eggs were formed.

We can now calculate the distances between y, m, and f. If we ignore the forked locus for the present, then the crossovers between y and m result in the combinations $+\,m$ and $y\,+$. In Fig. 59 these combinations are found in classes 3 and 4 (the single crossovers that take place in the y-m region) and in classes 7 and 8 (the doubles between y and f). Classes 3 and 4 are $15+15$ or 30 per cent of the total, and classes 7 and 8 are $3+3$ or 6 per cent of the total. Thus the total amount of crossing over between y and m is $30+6$ or 36 per cent. This makes the distance between y and m 36. The distance between m and f is got in a similar manner. The crossovers between m and f would be $+\,f$ and $m\,+$.

These combinations are found in classes 5 and 6 (the single cross-overs that occur in the *m-f* interval) and in classes 7 and 8 (the double crossovers). Classes 5 and 6 are $7 + 7$ or 14 per cent of the

F_1 FEMALE $\left(\dfrac{y\ m\ f}{+\ +\ +}\right)$
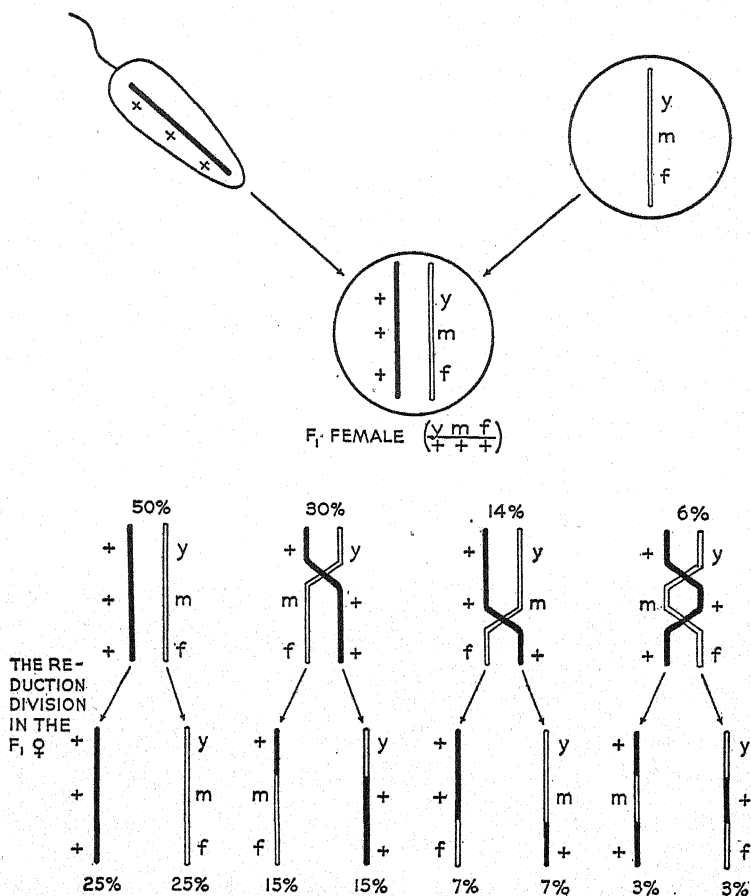
FIG. 59. A case of linkage involving three pairs of genes.

total, and classes 7 and 8 are $3+3$ or 6 per cent of the total. The total amount of crossing over between *m* and *f* is therefore $14 + 6$ or 20 per cent. This makes the distance between *m* and *f* 20. Since the order of the three genes under consideration is *y m f*, and since the two distances *y* to *m* and *m* to *f* are 36 and 20 respectively, the total distance between *y* and *f* is $36 + 20$ or 56.

It might seem a little confusing that we should count the double crossovers (classes 7 and 8) twice in getting the distances. But it should be borne in mind that a double crossover is the equivalent to two singles, one between y and m and another between m and f. The double crossovers are therefore counted twice in getting the total amount of crossing over between y and f.

If we confine ourselves to the y and f loci in Fig. 59, it will be seen that the double crossovers are $+ +$ and $y f$. But so are the non-crossovers. Hence, if we got a female of composition $\dfrac{y \quad f}{+ \quad +}$ and made a test cross, we could not distinguish the double crossovers from the non-crossovers, and so we should count them both together as non-crossovers. But the single crossovers would have resulted in recombinations between y and f ($y +$ and $+ f$). Thus only they would tell us of crossing over. Hence in the present experiment we should be gauging the per cent of crossing over by the per cent of *recombination* between y and f, and this would be the same as the per cent of the single crossovers. Now if we refer back to Fig. 59, we see that the singles form only $30 + 14$ or 44 per cent of the total crossovers. This would then be the *apparent* distance between y and f as obtained from a female hybrid at only the y and f loci $\left(\dfrac{y \quad f}{+ \quad +} \right)$. But the distance between y and f as obtained from a female hybrid at m as well as at y and f $\left(\text{one of composition } \dfrac{y \quad m \quad f}{+ \quad + \quad +} \right)$ was 56 ($36 + 20$). This is the *actual* distance. The difference between the actual and apparent distances (56 and 44) is due to the double crossovers. When we use a female of genotype $\dfrac{y \quad m \quad f}{+ \quad + \quad +}$ we observe and count the double crossovers, but when we use a female of genotype $\dfrac{y \quad f}{+ \quad +}$ we overlook the doubles, since the double crossovers now appear no different from the non-crossovers.

In a female of genotype $\dfrac{y \quad m \quad f}{+ \quad + \quad +}$ we gauge the two shorter distances (ym and mf) by the per cent of recombination between ym and between mf, and therefore we take into account only the single crossovers in getting the distance in each case. But within these

shorter distances there are comparatively few double crossovers. Hence the shorter distances are fairly accurately gauged by the per cent of recombination. But the longer distance *yf* is not because of the relatively large proportion of double crossovers. It is therefore obvious that the distance between *y* and *f* is obtained more accurately by adding together the two shorter distances than by getting the apparent distance *y* to *f* directly by the per cent of recombination between *y* and *f* (in a female hybrid at only *y* and *f*).

Determining the Order of Genes in a Chromosome.—In getting the distances between *y* and *m* and *f*, it would not be necessary to use a female hybrid at all three loci at the same time $\left(\text{such as } \dfrac{y \ m \ f}{+ + +}\right)$. A $\dfrac{y \ m}{+ +}$ female would give us the distance between *y* and *m* and a $\dfrac{m \ f}{+ +}$ female the distance between *m* and *f*.

In each case we should, of course, make the test cross and get the per cent of crossovers by counting the offspring. These would tell us that *y* and *m* were 36 units apart and that *m* and *f* were 20 units apart (36 and 20 being the per cents of crossing over between these loci, respectively). If now the order of the genes is *y m f*, then the distance apart of *y* and *f* is the sum of the distances *y m* and *m f;* that is, 36 + 20 or 56. But we do not yet know the order of the
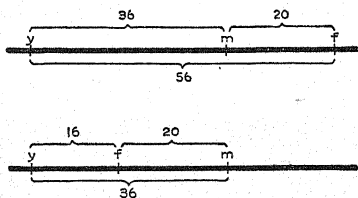
Fig. 60. Diagram showing the possible arrangements of three genes when only two of the distances are known.

genes; for all we know so far, *f* might be to the left of *m* instead of to the right (Fig. 60). In this case the distance *y f* would be 36 − 20 or 16 (instead of 56). In order to decide between the two possible orders, we should get a female hybrid for *y* and *f* $\left(\dfrac{y \ f}{+ +}\right)$ and determine the amount of recombination between *y* and *f*. From a female of this composition we should find that there was 44 per cent of recombination between *y* and *f*, and this represents the per cent of single crossing over between *y* and *f*. This figure is closer to 56 than to 16. It is not equal to 56 because it does not include the double crossovers, but it would

be approximately equal to 56 if it did include them. Therefore the order of the genes is $y\ m\ f$.

A test cross of a female hybrid at all three loci $\left(\dfrac{y\ \ m\ \ f}{+\ +\ +}\right)$ would have given us the distances between y and m, m and f, and y and f all at the same time, and so would have given us the order of the genes also. However, a female of this composition would have to be made up first, since it does not occur in nature.

In general, to determine the order of three linked genes a, b, and c, we get the distances between a and b, between b and c, and between a and c.

Chromosome Maps.—Figure 60 (upper part) shows the relative positions of $y\ m$ and f in the chromosome. Such a figure is known as a *chromosome map*. It is based on two assumptions: (1) that the genes are arranged in a line and (2) that the per cent of crossing over between two genes is an index of their distance apart. A chromosome map is in fact sometimes called a *crossover map*. It is constructed entirely from the results of breeding experiments (test crosses), not from microscopic examination of the chromosomes themselves. We might, then, define *a chromosome map* as *a line on which the genes are represented by points separated by distances proportional to the amount of crossing over*. The first two chromosome maps were made in 1911 by Sturtevant and soon after additional maps were made by Bridges and others, Drosophila being the earliest material employed.

Suppose now we had mapped y, m, and f and we wanted to add another gene to the map, say, carnation (*car*), an eye color gene

FIG. 61. The method of adding a new gene to a chromosome map.

in the X. We might go about locating carnation by first getting a female hybrid for forked and carnation $\left(\dfrac{f\ car}{+\ +}\right)$ and making a test cross. We should find that there was about 6 per cent crossing over between f and *car*, giving a distance of 6 between f and *car*. Since f is at 56, this would put *car* at either 62 or 50 (omitting decimals), depending on whether *car* was to the right or left of f (Fig. 61, upper part). To decide between these two possibilities, we should find the distance of *car*

from some other gene, say, *m* (miniature wings at locus 36). If *car* were at 62 then its distance from *m* would be 62 − 36 or 26; if at 50, then its distance from *m* would be 50 − 36 or 14. We should actually find that *car* was about 26 units from *m*, and therefore at 62 (Fig. 61, lower part). Thus in order to add a new gene to the map we must find its distance from at least two other genes on the map.

In Drosophila the positions of numerous mutant genes have been located in the chromosomes and maps made (Fig. 62). Drosophila has four pairs of chromosomes, three large and one small. These are numbered 1, 2, 3, and 4. One of the three large chromosomes is the *X*, and is designated as 1. The other two large chromosomes are the *V*-shaped autosomes, and are designated as 2 and 3. The very small chromosome is numbered 4.

In the case of the second chromosome of Drosophila, the two extreme genes are "aristaless" (*ar*, absence of the arista, a branch of the antenna) and "balloon" (*ba*, balloon-like blisters on the wing). Since the distance between these two genes is set down as 107.4, there must be 107.4 per cent of crossing over between them. That is to say, in every 100 gametes, there are 107.4 crossovers on the average. This may seem confusing until we remember that many of the gametes contain double and triple crossovers. If we count each double crossover twice (each triple crossover three times, etc.), and add these to the single, then there is no difficulty in getting a total of 107.4 crossovers on the average in every 100 gametes.

The per cent of *recombinations* between two pairs of genes in the same chromosome pair is always less than 50. This applies to a female hybrid for aristaless and balloon. If now we got a fly hybrid for aristaless and balloon only, and made a test cross, we should find that there were less than 50 per cent recombinations between aristaless and balloon, and so the length of the chromosome would be set down as less than 50 (instead of 107.4). Most of the recombinations would be due to the single crossovers (some to triples). But by means of intermediate loci we can detect the double crossovers. These result in no recombination between aristaless and balloon, but they add to the calculated distance between aristaless and balloon. Thus the length of the chromosome becomes 107.4.

Within a portion of a chromosome 10 units or less in length, there would be practically no double crossovers but only singles.
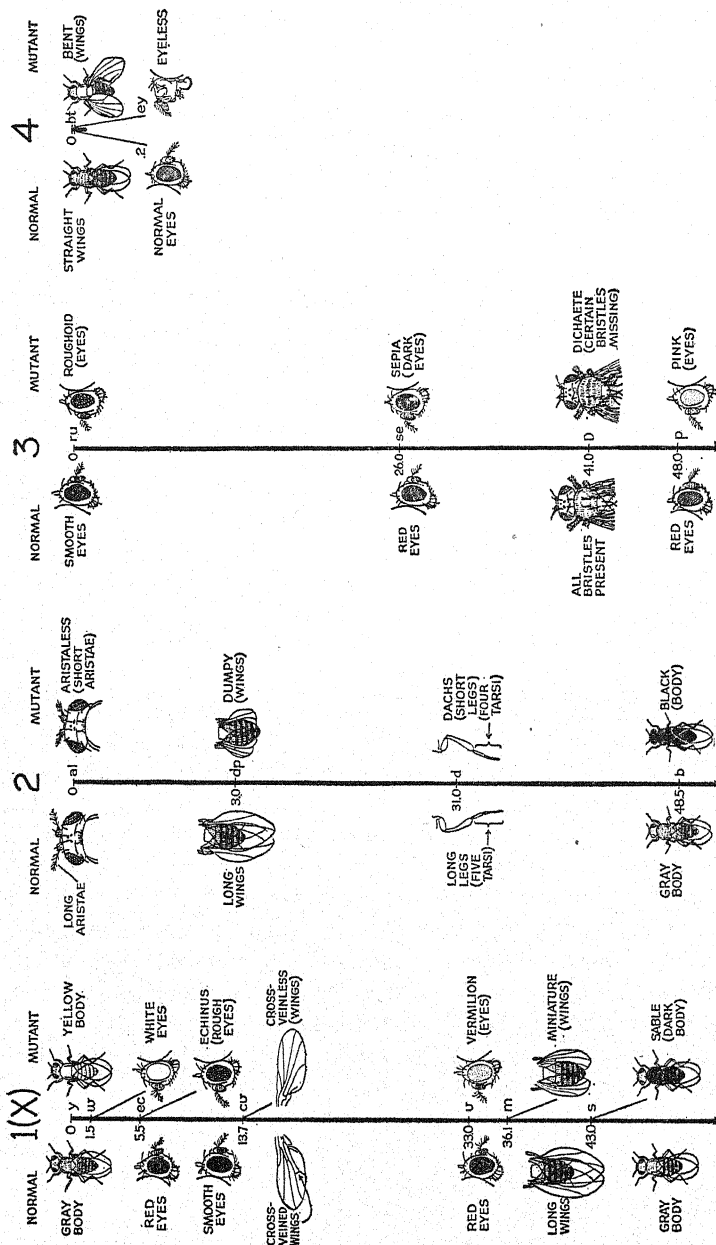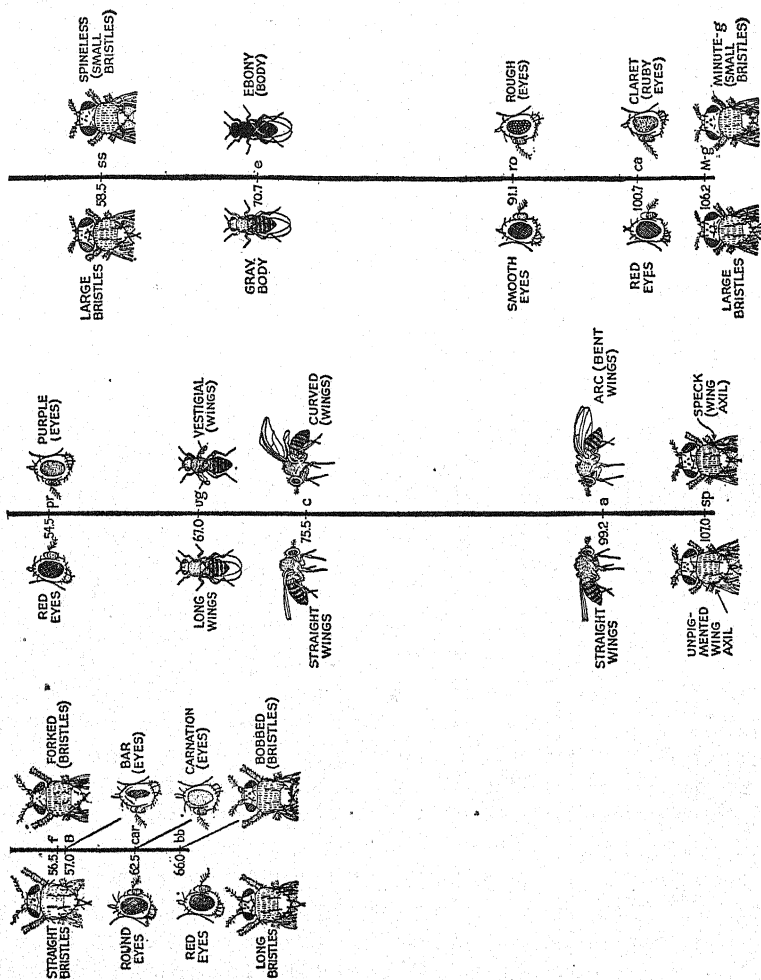
FIG. 62. Chromosome maps of *Drosophila melanogaster*. Only a few mutant genes, as compared to the total known number, are represented.
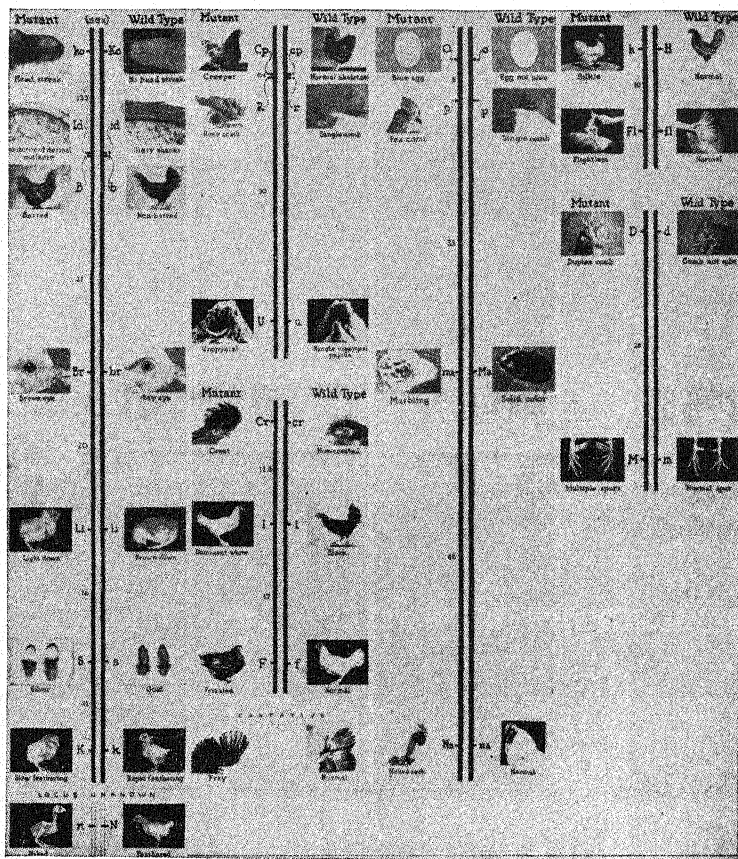
Fig. 63. Chromosome maps of the chicken. (From Hutt and Lamoreux in *The Journal of Heredity.*)

The distances between two genes less than 10 units apart would be accurately given by the singles, since there are no doubles (or very few). Therefore if we got the total length of a chromosome by means of genes not over 10 units apart, we could be reasonably sure that the total length was correctly gauged, since each of the smaller lengths was correct.

Chromosome maps have been made of other organisms besides Drosophila. Corn has been very intensively studied, and is second only to Drosophila in the number of genes that have been located on maps. Chickens have also been intensively studied (Fig. 63).

Genetic and Environmental Factors Which Influence Crossing Over.—The per cent of crossing over between two loci is not dependent entirely on their distance apart. In Drosophila crossing over is completely suppressed in the male, and as previously mentioned there is somewhat of a tendency for crossing over to be reduced in the heterozygous sex of many species. Also, mutations might reduce crossing over. In Drosophila Gowen has found a mutation which reduces crossing over in all the chromosomes. Sometimes crossing over is suppressed in a given segment of a chromosome. Usually, this is due to the accidental inversion of the segment in such a way that the order of the genes has been changed from a bc d, for example, to a cb d, so that in the hybrid $\left(\dfrac{a\ cb\ d}{a\ bc\ d}\right)$ crossing over within segment bc produces abnormal rearrangements, with the result that the crossovers (but not the noncrossovers) are lost. Then again, anything which interferes with the pairing of the chromosomes causes a reduction in crossing over. Finally, external agents might influence crossing over. In Drosophila, high and low temperatures tend to increase crossing over as compared with normal; age decreases it.

It is evident from the above that the per cent of crossing over between two loci is not an absolutely accurate index of their distance apart. But under uniform conditions, and on the average, the crossover per cent is a fairly accurate measure of distance. That this is true is shown by the fact that in Drosophila the relative lengths of the chromosomes as seen under the microscope and their relative map lengths agree.

Interference and Coincidence.—It is possible to figure out the per cent of double crossovers that are to be expected over a given length of chromosome. Possibly an analogy will help us to see how. Suppose that 10 per cent of the people in a certain community were tubercular and 20 per cent had blue eyes, and further that blue eyes had no connection with being tubercular. Then what per cent of the people in this community would be expected to be both tubercular and blue-eyed? Obviously, 10 per cent of 20 per cent, or 2 per cent. Just so with crossing over. If there is 10 per cent of crossing over between a and b, and 20 per cent between b and c, then the per cent of doubles expected on chance would be 10 per cent of 20 per cent, or 2 per cent. That is to say, two crossovers would happen to come together at the same time

in this proportion of cases, just as tuberculosis and blue eyes would, providing the one had nothing to do with the other and their coming together was just a matter of accident or *coincidence*. But when the distances between *a*, *b* and *c* are small, a crossing over between *a* and *b* prevents one between *b* and *c*, perhaps for the same reason as, in twisting two reeds about each other, their breaking might interfere with their looping back across one another near the break. The tendency of one crossover to suppress another in its vicinity is known as *interference*. One crossover is said to interfere with another one close by. It is because of interference that there are few or no double crossovers within a section of the chromosome 10 units or less in length. The amount of interference becomes less with increasing distance, and when the distance is long enough, then there is no interference. Thus, if one distance (between loci *a* and *b*) were, say, 40 and the other (between *b* and *c*) were also 40, then, ignoring for the sake of simplicity double crossovers occurring between *a* and *b* alone or between *b* and *c* alone, the proportion of double crossovers might be about what is expected (approximately 40 per cent of 40 per cent, or 16 per cent).

Double crossovers are the result of coming together or "coincidence" of two single crossovers. Hence when the doubles occur as often as expected on chance the coincidence is said to be 100 per cent. In this case the interference is 0. When there are no doubles, the interference is 100 per cent and the coincidence is 0. Coincidence is inversely proportional to the amount of interference.

Interference was discovered and named by H. J. Muller in 1911 (unpublished) from an analysis of data supplied by workers in the "Drosophila room" of Columbia University. Muller also proposed coincidence not long afterwards as an inverse measure of the amount of interference.

Proof of the Linear Arrangement of Genes.—Up to the present we have been *assuming* that the genes are arranged in a line. But their linear order can be shown by means of a cross involving three closely linked genes. The results of such a cross are graphically represented in Fig. 64, in which *a b c* represent three closely linked genes arranged in the order given. The break between *a* and *b* in the upper of the two lines in Fig. 64 represents a crossover between *a* and *b;* the break between *b* and *c* in the lower line represents a crossover between *b* and *c*. These are the

single crossovers and when distance *ac* is short, they are the only kinds of crossovers that occur between *a* and *c*, since there are no doubles. Hence whenever *a* or *c* separates from *b*, the two (*a* and *c*) separate from each other. The three genes can be compared to three links in a chain, the outer two links being held together by means of the middle link. In other words, the linkage of the genes is *linear*, and from this we conclude that the genes themselves are arranged in a line, or in linear order. Since genes that are close together are in linear order, those that are farther apart must also be, for they would be connected by intermediate genes that are closer together and that are in linear order.
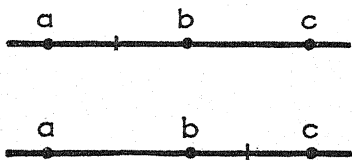
Fig. 64. Diagram to show the linkage of *a* and *c* through *b* (linear linkage).

Linear linkage is not as clearly shown in experiments involving longer distances, for in these we get double crossovers, and in the case of the double crossovers *a* and *c* remain together, although both of them separate from *b*. Thus it is not apparent that *a* and *c* are being held together by *b*. It is only when the distances are very short that *a* and *c* always separate from each other whenever either separates from *b*, because now there are no double crossovers. Hence experiments involving short distances prove that the linkage is linear. This fact was pointed out by Muller in 1912 (unpublished) following shortly upon his discovery of interference. Muller also pointed out that linear linkage could most simply be accounted for on the assumption that the genes themselves were arranged in a line.

Linkage and the Chromosome Theory of Heredity.—Mendel found that genes run in pairs. Later it was found that the chromosomes also run in pairs, and that the gene pairs and chromosome pairs have the same method of inheritance. It was therefore concluded that the genes were in the chromosomes. But if we examined the chromosomes of Mendel's pea plants under the microscope, we could not tell which one of them contained a particular gene. For all of the chromosomes run in pairs and which of the several pairs happens to contain, for example, the yellow-green pair of alleles is something we cannot tell by mere inspection of the chromosomes.

In many animals, however, including man, there is a chromosome

that does not have an exact mate in the male—the X chromosome —and all genes which show sex-linked inheritance, such as color blindness, are definitely known to be in the X. Evidence to this effect was first got in Drosophila. Miss N. M. Stevens found how the X was transmitted from the parents to the offspring in Drosophila, and Morgan then found that the mutant gene for white eyes was transmitted in the same way. It therefore followed that the gene white was in the X.

Other mutations were also found in the X, as for example miniature wings. But this in itself did not prove that the X contained more than one gene. For it would have been conceivable that the entire X chromosome of Drosophila represented just a single large gene and that white and miniature were mutations of this one gene in two different directions rather than mutations of two separate genes. But when a red long fly is crossed to a white miniature, some of the offspring from the test cross are white but not miniature (white long) and others are miniature but not white (red miniature). This proves that white and miniature are separable. Hence they are two separate genes. But white and miniature are both in the same chromosome (the X). Therefore one chromosome contains more than one gene. The evidence demonstrating that one chromosome contains more than one gene was first got by T. H. Morgan in 1911 by means of breeding experiments similar to those just described involving the separation of two genes in the X chromosome of Drosophila.

The linkage of white and miniature is most simply explained by the fact that the two genes are in the same chromosome. This explanation of linkage was first suggested by Morgan. The basis of the theory of crossing over was suggested by Janssens, a Belgian biologist, in 1909. Janssens observed that homologous chromosomes crossed each other at the time of the reduction division and concluded that they were exchanging segments. He referred to such crossings of chromosome strands as *chiasmata*, and the theory that the strands crossed as the result of an interchange of segments was known as the *chiasmatype theory*. Janssens used his chiasmatype theory to explain the supposed recombination between two pairs of alleles in the same chromosome pair, but that this occurs was not really proved until Morgan's work. Moreover, Morgan suggested that the amount of crossing over would be greater between genes further apart.

The work of Morgan and his colleagues Muller, Bridges, and Sturtevant did much to confirm the chromosome theory of inheritance, since it definitely placed the genes in the chromosomes and established their linear order.

SUMMARY

1. Homologous chromosomes often separate as a unit at the reduction division. But sometimes they break at corresponding loci and exchange corresponding segments. This is known as *crossing over*.

2. When two genes are close together in the same chromosome, they tend to remain together at the reduction division. They are said to be linked. But they might sometimes become separated by crossing over.

3. *Linkage* is the tendency of two genes to remain together in the process of inheritance.

4. If two genes (a and b) are in the same chromosome, then in the hybrid $\left(\dfrac{a\ b}{A\ B}\right)$ the per cent of the gametes with the old combinations (AB and ab) exceeds the per cent with the new combinations (Ab and aB). By contrast, when the genes are in separate chromosomes, the old combinations (AB and ab) and the new (Ab and aB) are equal.

5. The per cent of recombination between linked genes is different from one case of linkage to another and depends upon the distance apart of the genes, being greater the farther the genes are apart.

6. In Drosophila, black body (b) and vestigial wings (vg) are in the same chromosome (the "second") and are recessive to their normal alleles (for gray body and long wings). When a pure gray long is crossed to a black vestigial $\left(\dfrac{+\ +}{+\ +} \times \dfrac{b\ vg}{b\ vg}\right)$, the F_1 are $\dfrac{b\ \ vg}{+\ +}$. Females of this genotype produce eggs in the ratio of $41 + + : 41\ b\ vg : 9\ b + : 9 + vg$. The crossovers are $b +$ and $+ vg$. If the F_1 females are test crossed $\left(\dfrac{b\ \ vg}{+\ +}\ \female \times \dfrac{b\ vg}{b\ vg}\ \male\right)$, they produce offspring in the ratio $41\ \dfrac{+\ +}{b\ vg}$ (gray long) : $41\ \dfrac{b\ vg}{b\ vg}$ (black vestigial) : $9\ \dfrac{b +}{b\ vg}$ (black long) : $9\ \dfrac{+ vg}{b +}$ (gray long). The crossovers form 18 per cent of the total offspring.

7. In a female of genotype $\dfrac{b +}{+ vg}$ (in which $b +$ are in one chromosome and $+ vg$ in the other), the crossovers are $+ +$ and $b\ vg$, and they form

18 per cent of the total gametes, the per cent of crossing over being the same as in a female of genotype $\dfrac{b \quad vg}{+ \ +}$.

8. If we divide a chromosome into two equal halves, then there is, on the average, as much crossing over in one half as the other half, and twice as much over the entire length as over either half. Hence, if two genes are separated by the entire length of the chromosome, there is twice as much crossing over between them than if they are separated by only half the length. In general, the per cent of crossing over between two genes is proportional to their distance apart. Hence the per cent of crossing over between two genes is an index of their distance apart. If there is 1 per cent of crossing over between two genes, they are said to be 1 "unit" apart. If there is 18 per cent (as in the case of black and vestigial), they are 18 units apart.

9. There is no crossing over in the male of Drosophila (in which the male is the "heterozygous sex," or XY). Crossing over is not always completely absent in the heterozygous sex of other species, though it is often reduced.

10. By a "test cross" we mean a cross of a hybrid parent by a recessive $\left(\text{as } \dfrac{b \quad vg}{+ \ +} \ ♀ \times \dfrac{b \ vg}{b \ vg} \ ♂ \right)$. The offspring of such a cross show directly by their appearance what genes they receive from their hybrid parent, since they receive only recessive genes from their recessive parent. Hence the test cross directly tells us the per cent of crossing over in the hybrid parent.

11. In Drosophila the males have only one X and they receive this from their mother. Hence if the mother is heterozygous for two pairs of genes in her X-chromosomes $\left(\text{as } \dfrac{y \quad m}{+ \ +}\right)$, her male offspring are of the same four genetic classes as her eggs (as $+ \ +$, $y \ m$, $y \ +$, $+ \ m$), and so they tell us directly what the per cent of crossing over is in the hybrid mother. This is true regardless of the genotype of their father.

12. Sometimes crossing over occurs at two different points at the same time in a given pair of chromosomes. This is known as *double crossing over.*

13. In Drosophila, a female of genotype $\dfrac{y \quad m \quad f}{+ \ + \ +}$ forms eight classes of eggs: $+ \ + \ +$ and $y \ m \ f$ (non-crossovers), $y \ + \ +$ and $+ \ m \ f$ (singles between y and m), $y \ m \ +$ and $+ \ + \ f$ (singles between m and f), and $y \ + \ f$ and $+ \ m \ +$ (doubles between y and f).

14. In getting the per cent of crossing over between y and f, each double crossover is counted as the equivalent of two singles, and therefore each double crossover is counted twice, once in getting the distance between y and m and again in getting the distance between m and f, the doubles being added to the singles in each instance.

15. The genes $y\,m\,f$ are arranged in a line in the chromosome. Therefore, they can be represented as points along a line separated by distances proportional to the per cents of crossing over between them.

16. A *chromosome map* is a drawing in which linked genes are shown as points along a line, the points being separated by distances proportional to the per cents of crossing over between the genes, each point receiving its appropriate label.

17. Double crossing over between two genes (as y and f) results in no recombination between them.

18. By the "apparent" distance between two genes (as y and f) we mean the per cent of recombination between them. By the "actual" distance we mean the per cent of crossing over between them.

19. When there is double crossing over between two genes, the "apparent" distance between them is less than the actual, since the apparent distance does not take into account the double crossovers.

20. In the hybrid $\dfrac{y\;m\;f}{+\;+\;+}$ the non-crossovers $(+\,+\,+$ and $y\,m\,f)$ are the most frequent class of gametes, and the double crossovers $(+\,m\,+$ and $y\,+\,f)$ are the least frequent. Double crossing over removes the middle gene (m, or its normal allele) from the two outer genes. Hence if we are not told the order of the genes, we can determine this by comparing the non-crossovers (or most abundant classes) with the doubles (or least abundant), and so determine which gene is removed from its group by double crossing over. This is the middle gene.

21. The order of $y\,m\,f$ can be got (in a second way) by separately getting the apparent distances between ym, mf, and yf and putting the two genes represented by the greatest apparent distance (y and f) on the outsides.

22. Crossing over is influenced by (1) sex, (2) age, (3) temperature, (4) genetic constitution, and (5) chromosomal rearrangements which interfere with the normal pairing of the chromosomes at the reduction division.

23. The amount of crossing over tends to be equal over different chromosome segments of equal length, but there are exceptions to this rule.

24. When the distances between loci a and b and between b and c are small (less than about 5 units in each instance), then a crossover between a and b prevents a second, simultaneous crossover between b and c. In other words, one crossover prevents a second nearby crossover from occurring. This is known as *interference*.

25. When the distances involved are long (40 units or more in each instance), one crossover does not interfere with a second, simultaneous crossover, and the "coefficient of coincidence" is said to be 100 per cent. If double crossing over is, for example, half as frequent as expected on chance, the coefficient of coincidence is 50 per cent. In general, the coefficient of coincidence is the per cent that the actual crossovers are of the expected.

26. When the distances between a, b, and c are very small, there are no double crossovers between a and c (because of interference). Hence, whenever a and c separate from b, they separate from one another. This shows that a is linked to c *through* b. In other words, it shows that the linkage is *linear*. This in turn indicates that the genes themselves are arranged in linear order.

27. In Drosophila it has been proved that a chromosome contains more than one gene. Thus, for example, yellow and miniature are both in the X, because they have the same method of inheritance as the X (they are "sex-linked"). They are known to be two separate genes (rather than two mutations of the same gene), because they can be separated by crossing over.

28. The chromosome theory of inheritance was originally based on the discovery that Mendel's alleles and the chromosomes segregate in the same manner at the reduction division; but this theory was strongly confirmed when it was shown that a definite chromosome contains a definite group of genes, arranged in linear order.

PROBLEMS

1. In Drosophila pink eyes (p) are recessive to red ($+$); the absence of the larger bristles, referred to as "spineless" (s), is recessive to spined ($+$). Both pink and spineless are in the third chromosome. In a hybrid female of genotype $\dfrac{+\ +}{p\ s}$ there is 10 per cent of crossing over between p and s. Tell what four classes of eggs a $\dfrac{+\ +}{p\ s}$ female forms, giving the non-crossovers first and then the crossovers; and give the per cent that each class is of the total gametes. (Do not make each crossover class equal to 10 per cent of the total but both together.)

2. A pure red spined Drosophila is crossed to a pink spineless. Give the genotypes of the P_1 and the F_1. If an F_1 female is crossed to a pink spineless male, what four genotypic classes of offspring will be produced? What per cent will each class be of the total, and what will be their appearance? Tell what per cent the crossovers are of the total offspring and tell therefore what the "distance" between pink and spineless is judged to be.

3. A red spined Drosophila is crossed to a pink spineless, and the F_1 female is "test crossed" to a pink spineless male. The count of the offspring produced by the test cross is as follows.

Red spined. 453
Pink spined. 55
Red spineless. 50
Pink spineless. 442

Calculate the distance between pink and spineless as judged by this experiment.

4. A pure pink Drosophila is crossed to a pure spineless. Give the genotypes of the P_1 and the F_1. Give the non-crossover and the crossover classes of gametes and their per cents in the F_1 females (still assuming that there is 10 per cent of crossing over between the loci of p and s). Assume an F_1 female is crossed to a pink spineless male. Tell which classes of offspring are the non-crossovers and which the crossovers, giving both their genotypes and phenotypes, and the per cent each class is of the total offspring.

5. In corn white endosperm (w) is recessive to purple ($+$) and shrunken endosperm (s) is recessive to full ($+$). A pure purple shrunken is crossed to a pure white full. The F_1 is then crossed to a white shrunken, and the offspring are as follows:

Purple shrunken........................	3,149
Purple full............................	120
White shrunken........................	115
White full............................	3,334

Give the genotypes of the P_1 and the F_1 and give the crossover classes of gametes produced by the F_1. Calculate the distance between white and shrunken.

6. Given the hybrid $\dfrac{a \ \ b \ \ c}{+ + +}$ in which a and b are in the same chromosome and 10 units apart and c in a different chromosome. Give the four classes of gametes for loci a and b only, and their per cents. Next give the two classes of gametes for locus c only, and their per cents. Would these per cents apply within each of the first four classes of gametes (for loci a and b)? If so, then derive (by the "branching" method) the eight classes of gametes formed by the hybrid and give their per cents. Suppose this hybrid were test crossed $\left(\dfrac{a \ \ b \ \ c}{+ + +} \times \dfrac{a \ b \ c}{a \ b \ c} \right)$. Then tell what classes of offspring it would produce, and the per cent that each class is of the total offspring.

7. Tell from a direct inspection of the genotypes of the parents in the above test cross $\left(\dfrac{a \ \ b \ \ c}{+ + +} \times \dfrac{a \ b \ c}{a \ b \ c} \right)$ what four classes of offspring should be produced when each of the following three combinations of loci are separately considered: (1) a and b $\left(\text{so that the cross in effect is } \dfrac{a \ \ b}{+ +} \times \right.$

$\dfrac{a\,b}{a\,b}\Big)$, (2) a and c $\Big($cross in effect is $\dfrac{a}{+}\dfrac{c}{+}\times\dfrac{a\,c}{a\,c}\Big)$, (3) b and c $\Big($cross in effect is $\dfrac{b}{+}\dfrac{c}{+}\times\dfrac{b\,c}{b\,c}\Big)$.

8. If you were given only the count of the offspring from the above test cross $\Big(\dfrac{a\ b\ c}{+\ +\ +}\times\dfrac{a\,b\,c}{a\,b\,c}\Big)$, how would you determine that a and b were in the same chromosome and that a and c (and b and c) were in separate chromosomes? How many of the 8 classes of offspring produced by the test cross would contain the crossovers of classes $a +$ and $+ b$? How would you get the per cent of crossing over between a and b?

9. In Drosophila yellow body (y), cut wings (ct), and vermilion eyes (v) are three mutant genes in the X chromosome and are recessive, respectively, to gray body ($+$), non-cut ($+$), and red eyes ($+$). A yellow, cut, vermilion female is crossed to a normal male $\Big(\dfrac{y\,ct\,v}{y\,ct\,v}\ \female \times \dfrac{+\ +\ +}{}\ \male\Big)$. Give the F_1 genotypes and phenotypes.

10. Show the pairing of the chromosomes at the reduction division in a female Drosophila of genotype $\dfrac{y\ \ ct\ \ v}{+\ +\ +}$. (Simply represent the chromosomes as lines with labels for the genes to one side of them, in the order $y\,ct\,v$, with ct a little below the middle, if you are drawing the chromosomes in a vertical position.) Assume that in 69 per cent of the reducing cells the chromosomes pair without crossing over; that in 18 per cent there is crossing over between y and ct only (singles in region 1), in 11 per cent crossing over between ct and v only (singles in region 2), and in 2 per cent crossing over between both y and ct and between ct and v at the same time (doubles). Below each pair show the two contrary classes of eggs produced by that pair as a result of the reduction division, and give the per cent of each class. (Remember that the total must remain 100 per cent.) Give the total per cent of crossing over between y and ct. Be sure to include the per cent of crossing over in region 1 contributed by the doubles. Give also the total per cent of crossing over between ct and v and be sure to include the per cent contributed by the doubles.

11. Given the test cross $\dfrac{y\ \ ct\ \ v}{+\ +\ +}\ \female \times \dfrac{y\,ct\,v}{}\ \male$. Derive the offspring from this cross, giving both their genotypes and phenotypes (for females and males separately), and the per cent each class is of the total offspring. (Do not draw the chromosomes but simply give the symbols for each genotype, above and below a horizontal line.)

12. A yellow, cut, vermilion fly is crossed to a normal, and the F_1 females crossed to $y\,ct\,v$ males (the test cross). The offspring of the test cross are as follows.

(1) gray, non-cut, red $(+ + +)$................. 3,562
(2) yellow, cut, vermilion $(y\ ct\ v)$................. 3,425
(3) gray, cut, vermilion $(+ ct\ v)$................. 941
(4) yellow, non-cut, red $(y + +)$................. 884
(5) gray, non-cut, vermilion $(+ + v)$............. 592
(6) yellow, cut, red $(y\ ct +)$..................... 528
(7) gray, cut, red $(+ ct +)$...................... 107
(8) yellow, non-cut, vermilion $(y + v)$............ 96

Tell which of the above classes contain crossovers between y and ct and which between ct and v. Give then the total number of crossovers in each region, and figure the per cent these totals are of the total offspring. Give then the distance between y and ct and between ct and v, as shown by the experiment. Construct a chromosome map giving the locations of these three genes.

13. Assume that there is 10 per cent of crossing over between loci a and b and 15 per cent between b and c (the order of the loci being $a\ b\ c$). What would be the "expected" per cent of double crossovers, assuming that one crossover does not interfere with another? Suppose that the actual per cent (as experimentally determined) were 1 per cent. What would be the coefficient of coincidence?

14. Give the coefficient of coincidence in the experiment described in question 12.

15. In the test cross $\dfrac{y\ \ ct\ \ v}{+ + +}$ ♀ $\times \dfrac{y\ ct\ v}{}$ ♂, which two phenotypic classes of offspring are the most abundant? Are these crossovers or non-crossovers? If then you were given only the count of the offspring, how could you tell, from their relative numbers, that genes $y\ ct\ v$ were originally in one X of the hybrid parent and $+ + +$ in the other (the combination $\dfrac{y\ \ ct\ \ v}{+ + +}$ rather than some other combination, such as $\left.\dfrac{y + +}{+ ct\ v}\right)$?

16. In the Chinese primrose, long style (1) is recessive to short $(+)$, red flower color (r) is recessive to magenta $(+)$, and red stigma (rs) is recessive to green stigma $(+)$. Given the following count from a test cross.

Short style, magenta, red stigma.................... 290
Short style, magenta, green stigma................. 151
Long style, red, green stigma...................... 288
Long style, red, red stigma........................ 141
Short style, red, green stigma..................... 37
Short style, red, red stigma....................... 20
Long style, magenta, red stigma.................... 39
Long style, magenta, green stigma.................. 21

Give the phenotypes of the parents (the hybrid F_1 parent and the recessive parent to which it was test crossed). Tell which classes contain the crossovers between l and r (region 1) and which between r and s (region 2). Next calculate what per cents the crossovers in each region are of the total offspring and give the distance between l and r and between r and s. Construct a chromosome map giving the locations of three genes.

17. In the test cross $\dfrac{y \ \ ct \ \ v}{+ \ + \ +} \times \dfrac{y \ ct \ v}{}$, which two classes are the least numerous? Which gene is removed from the $y \ ct \ v$ chromosome by double crossing over (middle or one of the end genes)? If then you were given the three genes in the wrong order, say, $ct \ y \ v$, but were told that one of the non-crossover classes was $ct \ y \ v$ and one of the double crossover classes was $+ \ y \ v$, what would the order of the genes be? Could you have drawn the same conclusion if you had been told that one of the non-crossover classes was $+ \ + \ +$ and one of the doubles was $ct \ + \ +$?

18. Given the test cross $\dfrac{+ \ ct \ \ v}{y \ + \ +} \times \dfrac{y \ ct \ v}{}$. Tell which the non-crossovers are and which the doubles, among the offspring. (Use the gene symbols to indicate the offspring.)

19. Given a test cross in which the two non-crossover classes of offspring are $+ \ y \ v$ and $ct \ + \ +$ and the two double crossovers $ct \ y \ v$ and $+ \ + \ +$. Tell with which double crossover class you would compare class $+ \ y \ v$ to get the correct order of the genes. Would the other two classes (those contrary to the ones you used) have done equally well? Give now the genotype of the hybrid parent with the genes in their correct order.

20. In Drosophila black body (b) is recessive to gray ($+$), vestigial wings (v) are recessive to long ($+$), and purple eyes (p) to red. (The dominant traits are those possessed by the normal flies, the recessive traits are mutants. Flies are usually named according to their mutant traits. Thus, flies designated as black in the cross below are normal as regards wings and eye color and are therefore black, long, red, and in gene symbols would be indicated as $b \ + \ +$. The wild type or normal is gray, long, red, or $+ \ + \ +$.) Given the following count of offspring from a back cross.

Black	72
Black, vestigial	381
Black, purple	43
Black, vestigial, purple	2
Purple	464
Vestigial	35
Vestigial, purple	55
Wild type	2

Tell which classes are the non-crossovers and put down the genotypes of the hybrid parent, giving for the present just the original combinations of genes in each chromosome (the non-crossover combinations), without reference to their order. Next, determine which classes of offspring are the double crossovers (from an inspection of the count) and get the correct order of the genes (from a comparison of either non-crossover class with the double that has two genes in common with it). Finally, get the per cents of crossing over in region 1 and region 2, and construct a chromosome map.

11. *CROSSING OVER AND MEIOSIS*

THE REDUCTION division is very important in heredity, because through it the chromosomes are properly distributed to the sperm and egg cells. But the essential thing at the reduction division is the pairing of the chromosomes. It is this that results in the reduction of the chromosome number. It is this too that results in the segregation of alleles and in Mendelian recombination. Finally, it is during the pairing of the chromosomes that crossing over takes place. The pairing of the chromosomes at the reduction division is obviously important and has received a special name. It is known as *synapsis*.

Meiosis.—It will be recalled that at the reduction division the chromosomes not only pair but they also split, as shown in Fig. 65, in which we begin with three pairs of chromosomes (long, medium, and short). The two split halves of each chromosome are known as *chromatids*. The pairing and splitting result in a group of four chromatids, known as a *tetrad*. The splitting, however, does not as yet involve the centromeres (the bodies to which the spindle fibers become attached, shown as dots at the ends of the chromosomes in Fig. 65), and we may still regard the two chromatids held together by a given centromere as a single, though split, chromosome. Crossing over now takes place, but a given crossing over involves only two of the four chromatids of a tetrad. Moreover, it always involves two chromatids not connected by the same centromere (non-sister chromatids). Next the chromosomes separate. This involves the separation of homologous centromeres (those attached to homologous chromosomes). Hence, the chromosome number is reduced (in Fig. 65 from three pairs to three single). But a given chromosome still consists of two chromatids held together by a centromere. The two chromatids in question are referred to as a *dyad*.

The equation division now takes place. This is preceded by the division of the centromeres. The chromosome number is not further reduced by the equation division, if we regard the two

THE REDUCTION DIVISION

SYNAPSIS

TETRADS

CROSSING OVER

THE EQUATION DIVISION

DYADS

GAMETES
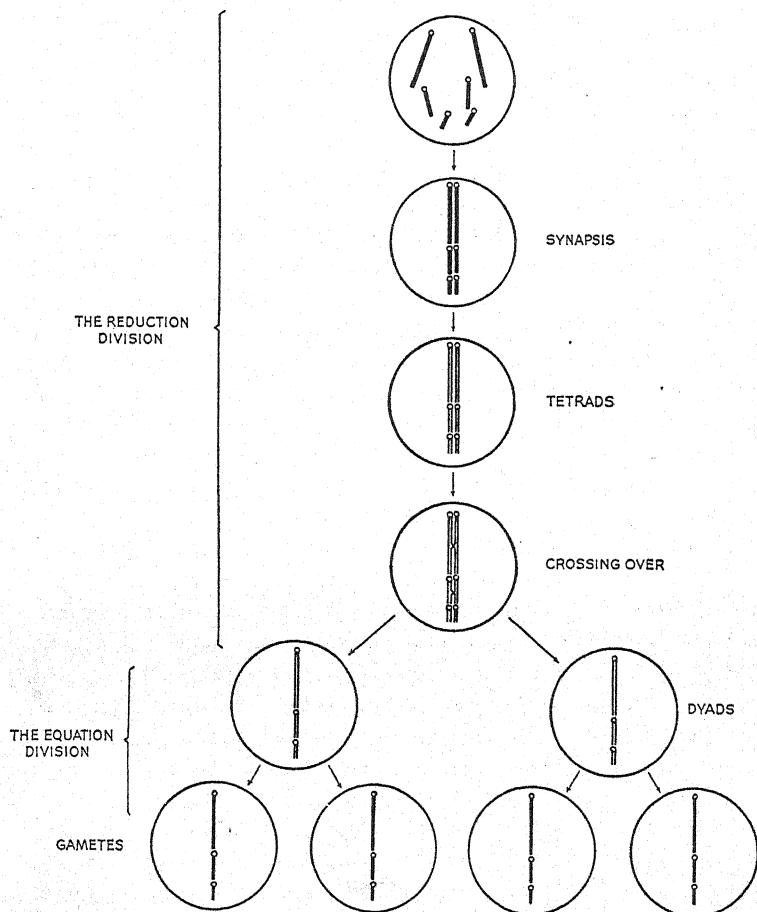
FIG. 65. The reduction and equation divisions, including tetrad formation and crossing over.

chromatids originally held together by a single centromere as one chromosome. Thus, in Fig. 65 the equation division results in four cells each of which still has three chromosomes, the same number as before the division, but each chromosome now consists of a single strand.

It is evident that the reduction and the equation divisions are intimately connected. The changes which the chromosomes undergo during these two divisions are known as *meiosis* (my-o'sis). They include the pairing and splitting of homologous chromosomes, crossing over, the separation of homologous chromosomes or their centromeres (reduction), and the separation of their split halves (at the equation division). The two cell divisions in which these changes occur are often referred to as the first and second *meiotic* divisions. The first meiotic division is the reduction division; the second, the equation division. The term *meiosis* in Greek means a belittling or making smaller, and it refers to the reduction in chromosome number in the present connection.

The sperm and egg cells mature during the two cell divisions under discussion (reduction and equation, or first and second meiotic) and these divisions are therefore sometimes referred to as the first and second *maturation* divisions. Thus we might use either of three sets of terms to apply to the two cell divisions that precede the formation of the sperm and egg cells: namely, (1) reduction and equation divisions, (2) first and second meiotic divisions, (3) first and second maturation divisions. The terms "first and second meiotic divisions" have come into general use.

The two meiotic divisions result in four cells, and in the male all four develop into sperm cells. But in the female only one develops into an egg; the rest form the small degenerate polar bodies. The formation of the polar bodies is connected with nutrition. Egg cells contain a store of food for the young, and just one cell in four receives all the available food. The remaining three degenerate and are the polar bodies. Sperm cells do not store food. Instead they are small and are formed in large numbers. Their small size increases their motility, and their large number increases their chances of reaching the egg and fertilizing it.

Crossing Over and Chiasmata.—Figure 66 shows in detail how crossing over takes place. First the chromosomes pair (Fig. 66-1), then they split to form a tetrad (Fig. 66-2). The chromatids that are shaded alike in Fig. 66-2 are derived by division

FIG. 66. Crossing over and chiasma formation.

from the same mother chromosome and are called sister chromatids. When crossing over takes place, two *non*-sister chromatids

AS CONVENTIONALLY REPRESENTED   AS ACTUALLY HAPPENS
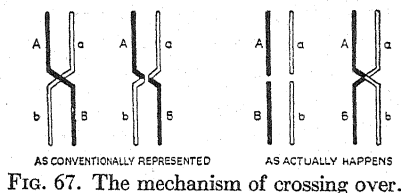
Fɪɢ. 67. The mechanism of crossing over.

first break at corresponding points (Fig. 66-3). Next a segment on one side of each break becomes connected with a segment on the opposite side of the other break (*A* with *b* and *a* with *B* in Fig. 66-3). As a result the two chromatids now cross each other. The crossing of two chromatids as a result of crossing over is known as a *chiasma* (the Greek word for cross, the plural of which is *chiasmata*).

Heretofore we have represented crossing over as though it involved first the crossing of two chromosomes (Fig. 67, left). Next breakage was supposed to occur and then union of the broken ends. This is just a convenient way of showing crossing over, but it is really not correct. For actually the chromosomes break *before* they cross, not after (Fig. 67, right). Moreover, the crossing of the chromosomes (a chiasma) is the *result* of crossing over, not the cause of it. If Fig. 67, left, were correct, then crossing over would unmake a chiasma; actually, it *makes* a chiasma (Fig. 67, right).

Crossing over may occur at several levels in a given tetrad, resulting in several chiasmata. The number of chiasmata varies with chromosomes of different length, but for chromosomes of average length, the number per tetrad is one to three.

Fɪɢ. 68. Terminalization.

Terminalization.—After chiasma formation, non-sister chromatids repel each other and tend to fall apart (Fig. 68, a and b).
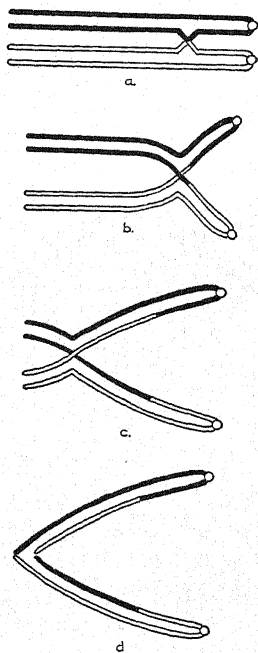
The centromeres especially repel each other, and this causes the chromatids to separate progressively from the centromeres towards a chiasma (Fig. 68b). Here their separation causes the chiasma to move towards the end of the tetrad, in zipper fashion (Fig. 68,

a. THE FORMATION OF A
DOUBLE X OR A RING

b. THE FORMATION OF AN
X, A V, OR AN I
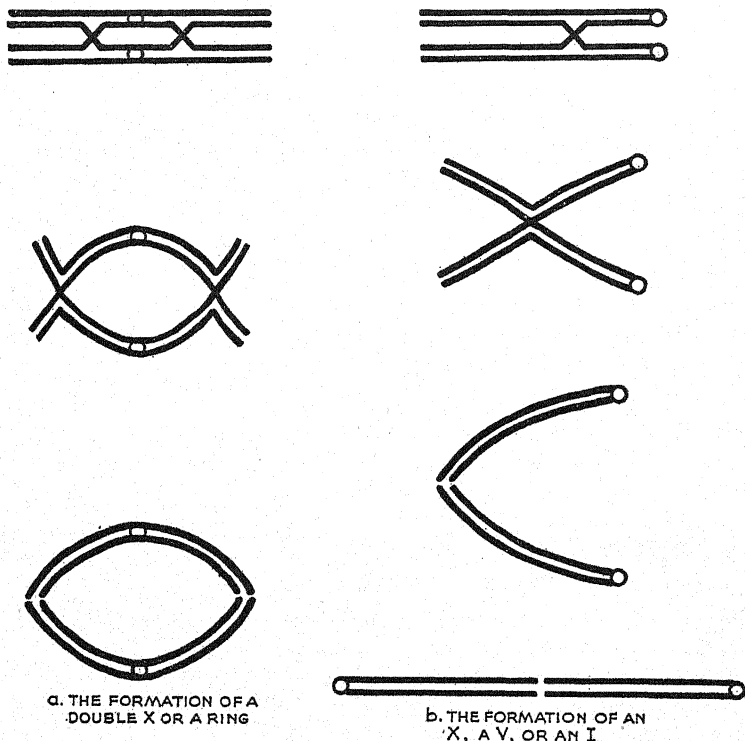
Fig. 69. Changes in the shapes of tetrads.

c and d). The movement of a chiasma away from the centromere and towards the end of a tetrad is known as *terminalization.*

In a tetrad, sister strands are at first associated (Fig. 68, a and b), but in the process of terminalization sister strands become separated and non-sister strands become associated (Fig. 68c). When terminalization is completed, non-sister chromatids are connected at their non-centromere ends (Fig. 68d). Terminalization may be incomplete in that a chiasma may move only part way to the end of a tetrad. When there are several chiasmata between a

centromere and the end of a tetrad, complete terminalization of the chiasma nearest the centromere eliminates the rest.

The exact shape of a tetrad after terminalization depends in part on the number of chiasmata it contains and on the extent of terminalization. It depends also on the position of the centromere. Suppose, for example, that the centromere were located in the middle of the chromosome and that there were a chiasma on each side of the centromere, as shown in Fig. 69, left. Then the tetrad would form a double X or a ring, depending on the completeness of termination. Suppose on the other hand that the centromere were terminal and that there were one chiasma as shown in Fig. 69, right. Then the tetrad would form an X or a V, depending on the extent of terminalization. The arms of the V might open up completely and form a straight rod (with an apparent transverse division where the chromosomes are still attached end to end). In any event the tetrad eventually forms two dyads.

Crossing Over and the Reduction Division.—Figure 70 shows how the four chromatids of a tetrad are distributed to the sperm or egg cells, when crossing over is taken into account. After crossing over takes place, all four strands are different as regards their origin, as shown by the fact that no two chromosomes are shaded alike in Fig. 70 (bottom). There are no longer two like sister strands derived from the splitting of each homologous chromosome, and, strictly speaking, the reduction division does not separate the two homologous chromosomes from each other; that is to say, it does not separate the two original dark chromatids in Fig. 70 from the two light. However, after crossing over takes place, the chromatids are still held together by a single centromere, and the two chromatids thus associated can still be considered as the equivalent of one chromosome. The division under discussion can therefore still be regarded as the reduction division, insofar as it separates the equivalent of two homologous chromosomes from each other. In Drosophila there is no crossing over in the male, and in this case the division under discussion is, strictly speaking, a reduction division, since homologous chromosomes unmodified by crossing over are separated from each other. Some geneticists however refer to the first and second meiotic divisions together as the reduction division*s*.

The Germ Cells of Animals.—The sperm or egg cells of an animal are derived eventually from the fertilized egg through the

process of cell division. There is thus a long series of cells which are ancestral to the sperm and egg cells. These ancestral cells,
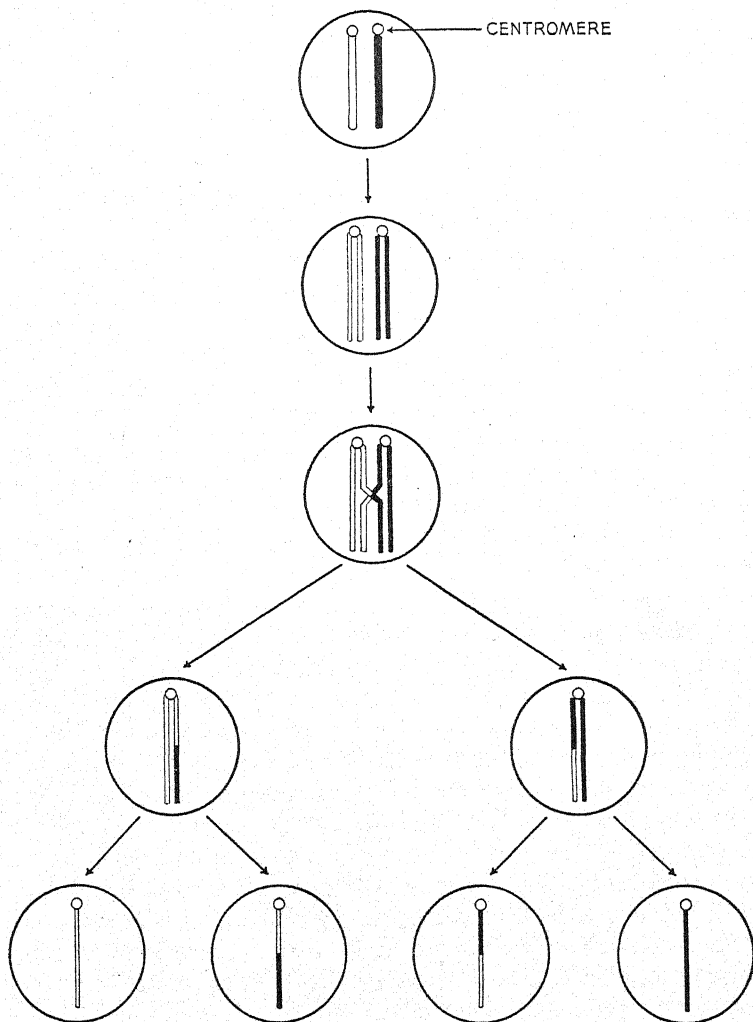
FIG. 70. The distribution of the products of a tetrad.

together with the sperm and egg cells proper, are referred to as the *germ cells* (or the cells of the *germ track*). It is important that we do not use the term "germ cells" to mean the same thing as

"sperm and egg cells," since the germ cells include not only the sperm and egg cells but also all cells ancestral to them.

In the very early embryo, the ovaries or testes are not yet developed, and the germ cells are undifferentiated cells not markedly different in appearance from other embryonic cells; but they are localized in a definite region of the embryo. They are known as the *primordial germ cells* (Fig. 71). They contain the unreduced number of chromosomes, and they multiply by mitotic cell division, just as do ordinary cells. As the embryo develops, its reproductive organs eventually are formed; but at first the germ cells are still undifferentiated and they still multiply by mitotic cell division and continue to contain the unreduced chromosome number. The reproductive organs are often referred to as *gonads* and the undifferentiated germ cells under discussion are known as *gonial cells*. In the testes the gonial cells are referred to as *spermatogonial cells* (Fig. 71). The gonial cells eventually give rise to the cells that undergo the reduction division, or first meiotic division. In the testis these latter cells are known as the *primary spermatocytes* (Fig. 71).

The nucleus of a primary spermatocyte is at first of ordinary size, but it grows and becomes much larger than the nucleus of a gonial cell. The time during which the nucleus of the primary spermatocyte grows is known as the *growth period*. It is during the growth period that the pairing and splitting of the chromosomes takes place, resulting in the formation of the tetrads. All this takes place during the early stages of the reduction division, before the tetrads get to the middle of the dividing cell. In other words, it all takes place during the *prophase* of the reduction division. At the completion of the growth period the tetrads line up in the middle of the cell. This represents the metaphase in the division of the primary spermatocyte; that is to say, it represents the metaphase of the reduction division in the male. The metaphase is followed by the anaphase and telophase and the division of the primary spermatocyte is completed. By its division the primary spermatocyte gives rise to the *secondary spermatocytes*. These are the cells that contain the dyads and that undergo the equation division. They give rise (through the equation division or second meiotic division) to the immature sperm cells, or *spermatids*. Finally, the spermatids differentiate into the *sperm cells*.
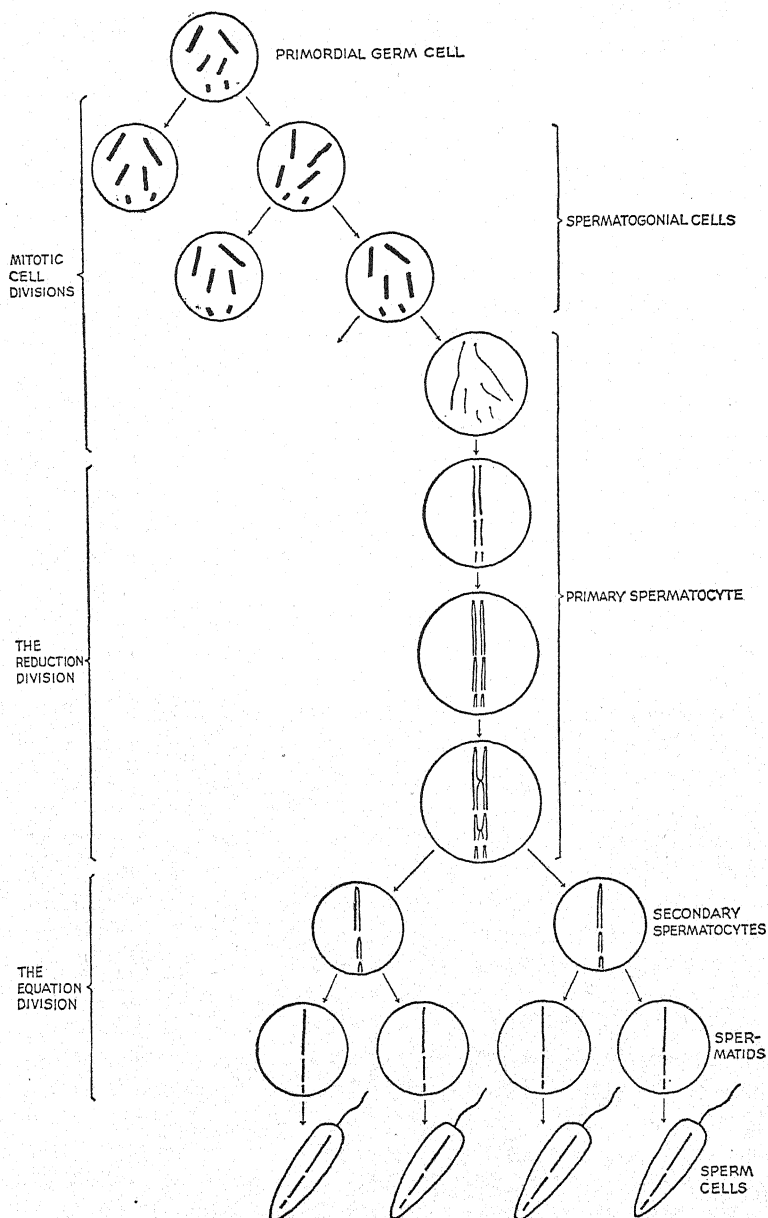
Fig. 71. The germ track (in the male).

In summary, then, the germ cells of the early embryo are called *primordial germ cells*. In the testes, the germ cells in the order of their succession are the *spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids*, and *sperm cells* (Fig. 71). The corresponding terms for the germ cells in the ovary are the *oögonia, primary oöcytes, secondary oöcytes, oötids*, and *eggs*.

In the male of most animals sperm cells continue to be formed throughout the life of the individual in large numbers, and hence the testis always contains large numbers of cells undergoing the reduction division—the primary spermatocytes. In the female of many animals the eggs are all formed at an early period in life and at most times it is difficult to find primary oocytes. Hence for studies of the reduction division in animals the testes with their numerous primary spermatocytes are preferred.

The Germ Cells of Plants.—The germ cells of plants correspond in a general way with those of animals. The early cells of the germ track are derived from the fertilized egg and multiply by mitosis. As the plant gets older it forms its reproductive organs— the anthers (which produce the pollen cells or microspores) and the pistils (which produce corresponding cells known as megaspores). Within the young anthers there are cells known as *pollen mother cells*. These give rise by two successive cell divisions to the pollen cells. The first is the reduction division; the second, the equation division. Thus from each pollen mother cell four pollen cells are formed, and these have the reduced chromosome number. The pollen cells are not sperm cells proper, but they give rise to the sperm cells by cell division (at the time the pollen tube grows down the pistil). In the young pistil there are cells known as *megaspore mother cells*. They give rise to the megaspores by two successive divisions (reduction and equation). Only one of the four cells formed by a megaspore mother cell develops into a megaspore. The megaspores eventually give rise to the egg cells.

In plants the pollen cells are formed in large numbers (as compared to the megaspores), and the young anthers contain numerous pollen mother cells. For studies of the reduction division in plants the young anthers are usually selected and the pollen mother cells are examined.

The Detailed Stages of Meiosis.—Meiosis is a complicated process and involves some details not already considered. The

description which follows might apply to either plants or animals, in favorable cases.

At the beginning of the reduction division the chromosomes condense from the resting nucleus as long thin threads, and at

a LEPTOTENE

b. ZYGOTENE
(OR AMPHITENE)

c. PACHYTENE

PROPHASE OF
FIRST MEIOTIC
DIVISION
(GROWTH PERIOD)

d. DIPLOTENE
(EARLY)

e. DIPLOTENE
(LATER)

f. DIAKINESIS

g. METAPHASE OF
FIRST MEIOTIC
(REDUCTION) DIVISION

h. ANAPHASE OF
FIRST MEIOTIC
DIVISION

i. ANAPHASE OF
SECOND MEIOTIC
(EQUATION)
DIVISION
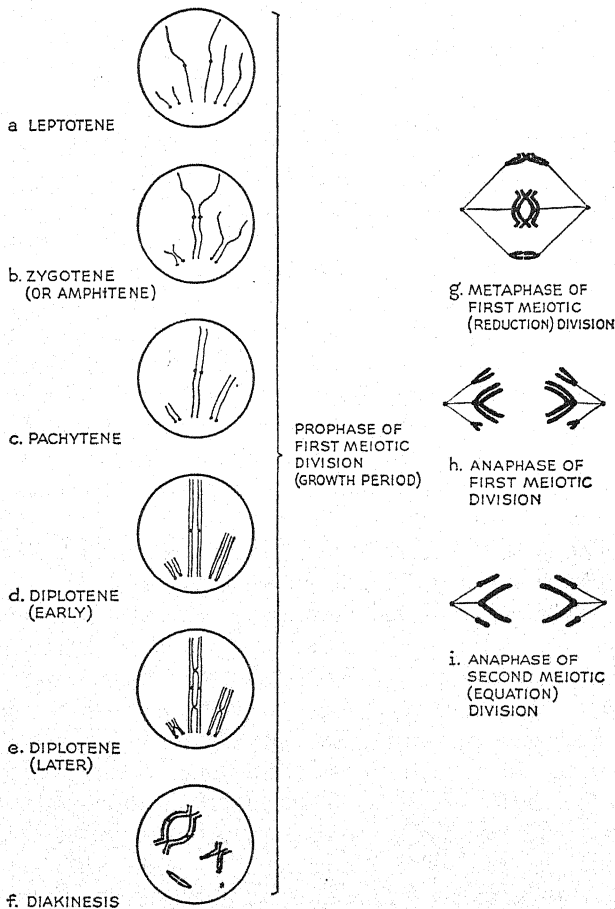
FIG. 72. The stages of meiosis in detail (diagrammatic). The circle surrounding the chromosomes represents the nuclear wall (not the cell wall).

first they are unpaired (Fig. 72a). This stage in meiosis is known as the *leptotene* stage. Somewhat later the chromosomes begin to pair and they are now said to be in the *zygotene* stage (Fig. 72b).

Often the pairing begins at one end of the chromosome pair and proceeds in zipper fashion to the other end. The chromosomes get shorter and thicker during zygotene and they continue to do so during the later stages of the growth period. At the end of the zygotene two homologous chromosomes have come so close together that no distinct line of separation can be seen between them, and they are now said to be in the *pachytene* stage (Fig. 72c). The splitting of the chromosomes probably takes place in the pachytene stage, but it is difficult to see the actual cleavage. Next the chromosomes become visibly split and tetrads are formed. Also crossing over takes place and chiasmata are formed. This stage in meiosis is referred to as the *diplotene* stage (Fig. 72d, e). By the end of the diplotene the chromosomes have got short and thick and they form ordinary tetrads. Next the tetrads assume various shapes because of terminalization. At the same time they move outwards until they come into contact with the wall of the nucleus (which is still intact). This stage in meiosis is known as *diakinesis* (Fig. 72f).

The nuclear wall next breaks down, and the chromosomes are free to move about in the general cytoplasm. Everything up to this point happens during the growth period—the prophase of the first meiotic division. At the end of the prophase the tetrads have arrived in the middle of the cell and are attached to spindle fibers from opposite poles of the cell. This constitutes the *metaphase* stage of the first meiotic division (Fig. 72g). The chromosomes are then drawn to opposite poles of the dividing cell (*anaphase* and *telophase*) and the first meiotic division is completed by the formation of a cell wall between the separated sets of chromosomes. Next the second meiotic division takes place and the sperm or egg cells are formed.

Figure 73 shows some of the stages in the reduction division as they actually appear under the microscope. The material illustrated in this figure is taken from an insect (Chorthippus). In plant material the chromosomes often become tightly bunched up during pachytene. This bunching up is referred to as *synizesis*. Perhaps it is produced in part by the chemicals used in preparing the specimens for microscopic examination.

The Precocity Theory.—According to Darlington, meiosis can be related to mitosis as follows. In *mitosis* the chromosomes split during the resting stage, before they are distinctly visible as

threads. The split halves, or sister chromatids, remain together until metaphase because they are alike and like chromosomes or chromatids attract one another in pairs. The sister chromatids do not attract their homologues (formed by the splitting of the homologous chromosome) because the affinity of each chromatid for a like chromatid has already been satisfied. Now in *meiosis* the

a. LEPTOTENE   b. ZYGOTENE   c. PACHYTENE
                (OR AMPHITENE)
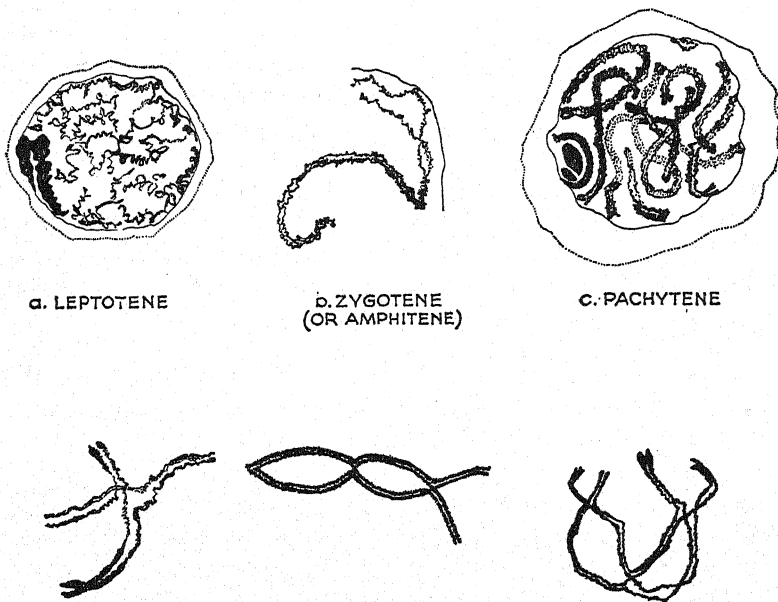
d. DIPLOTENE, SHOWING CHIASMATA (TETRADS SELECTED FROM THREE NUCLEI)

FIG. 73. The stages of meiosis as seen in the insect *Chorthippus parallelus.* (From Janssens, in *La Cellule.*)

chromosomes condense out of the resting nucleus *before* they have had time to split and therefore homologous chromosomes attract one another and pair. Next they split just as they would in mitosis, thus forming a tetrad. Sister chromatids now attract each other (because they are closer together than non-sister chromatids). But after a chromatid has satisfied its attraction for a like chromatid it repels other like chromatids. Hence one pair of sister chromatids now repels the other pair and the tetrad tends to fall apart into two dyads. But the chiasmata hold together homologous chromatids. Hence wherever there are chiasmata the chromosomes do

not fall apart, but in the intervals between chiasmata they do. Terminalization now takes place, followed by the separation of the chromatids into dyads.

On Darlington's theory the main difference between meiosis and mitosis hinges on the simple fact that in meiosis the chromosomes condense *precociously* out of the resting nucleus and therefore they pair. Darlington's explanation of meiotic pairing (or synapsis) is therefore known as the precocity theory.

The Strain Theory of Crossing Over.—Darlington has attempted to account for crossing over as follows. When two homologous chromosomes start to pair they wind around each other (Fig. 74). This is known as *relational coiling* (the cause of which is not exactly understood). As a mechanical result of relational coiling, each of two pairing chromosomes itself becomes spirally wound up, and this is referred to as *spiralization*. Relational coiling is tight and exerts a strain on the chromosomes. So long as the chromosomes are not split they are strong enough to stand the strain, but after they split each one forms two weaker chromatids, with the result that a chromatid breaks somewhere along the line. This releases the strain on the sister chromatid but it somehow increases the strain on the non-sister chromatids and causes a break in one of them at a point opposite the first break (between the same loci). The broken chromatids now unwind near their breakage points, and in so doing they come into contact at their broken ends and unite, thus forming a chiasma. At the same time the strain on the chromosomes is locally released; hence there is no further breakage in the vicinity of the chiasma. Thus one chiasma prevents another in its immediate vicinity. This constitutes interference.
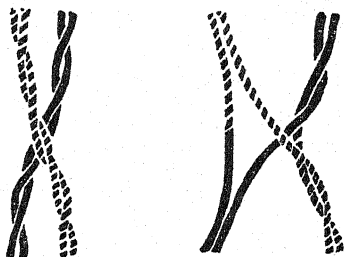
Fig. 74. Relational coiling and chromosome breakage. (From Darlington in *The Journal of Genetics*.)

Cytological Evidence of Crossing Over.—Suppose that we performed a linkage experiment and then examined the offspring under a microscope for their chromosomes. We should as a rule find that the chromosomes of the crossover classes appeared no different from those of the non-crossovers, because crossing over

normally produces no permanent, visible alteration in the structure of a chromosome; that is to say, it produces no cytological change. It is possible however to devise experiments in which we can get cytological evidence of crossing over. An experiment by Stern might be given in illustration.

In Drosophila the normal race has red round eyes. The mutant genes carnation (*car* or light red eyes, recessive) and Bar (*Ba* or

FEMALE WITH MODIFIED X'S AND OF GENOTYPE $\frac{car\ \ Ba}{+\ \ +}$

THE REDUCTION DIVISION IN THE ABOVE FEMALE

X-SPERM OF
car + MALE

CARNATION BAR   RED ROUND   RED BAR   CARNATION ROUND

NON-CROSSOVERS   CROSSOVERS
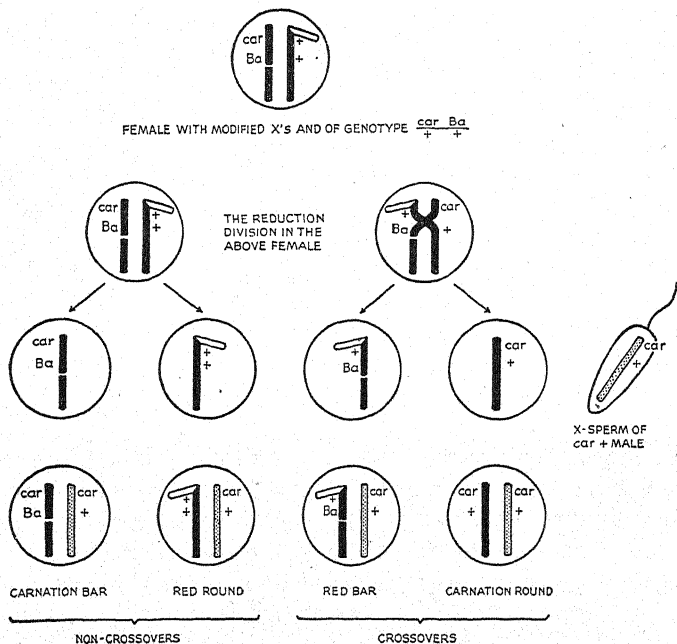
FIG. 75. Cytological proof of crossing over.

narrow eyes, dominant) are both in the X chromosome. The X chromosome of Drosophila is normally rod-shaped and a female normally contains a pair of these rod-shaped X's. But in Stern's experiment a female is obtained in which one X is broken into two (as the result of X-ray treatment) and in addition contains the mutant genes *car* and *Ba* (Fig. 75). The other X has a fragment of the Y chromosome attached to one of its ends (again as the result of X-ray treatment) and in addition contains the normal alleles (+'s) of carnation and Bar. One fragment of the broken X

has the centromere which belongs to the X and the other fragment has a centromere which was broken off of another chromosome (the fourth) as the result of X-ray treatment. Since both fragments have a centromere, they are distributed in the normal manner in cell division. The fragment of the Y contains no genes to speak of and in no way interferes with development.

The object in having the X's changed as compared to the normal is to distinguish the crossovers from the non-crossovers microscopically, as can be seen by inspection of Fig. 75. Crossing over between *car* and *Ba* results in the broken X having the fragment of the Y and in the unbroken X not having it. Before crossing over the opposite is true. Thus the crossover chromosomes are microscopically different from the non-crossovers.

In Fig. 75, bottom, an X chromosome containing *car* $+$ is added to each of the four classes of eggs produced by the hybrid female. This gives us her female offspring from a test cross. When the chromosomes of these offspring are examined under the microscope, the carnation Bar (first class in Fig. 75) are found to have the broken X without the fragment of the Y, and the red round (second class) have the unbroken X with the attached Y fragment. The carnation round (third class) have the unbroken X without an attached Y fragment, and the red Bar (fourth class) have the broken X with an attached X fragment. In other words, when the two *genetic* non-crossover classes are examined under the microscope, they are seen to contain the non-crossover X's of the mother, but the two crossover classes are seen to contain the crossover X's. This constitutes cytological proof of crossing over.

Proof of Crossing Over at the Four-strand Stage.—We can think of two rather distinct stages in the prophase of the reduction division. First the chromosomes pair, then they split. While they are paired but not yet split, a pair of chromosomes forms two strands; after they split the pair forms four strands (the four chromatids of a tetrad). Since the chromosomes are paired at the two-strand stage, crossing over might theoretically take place at that stage; but the experimental evidence shows that *crossing over takes place at the four-strand stage*. The evidence involves the use of an abnormal stock of flies. It will be recalled that normally only one of the chromosomes of a given tetrad goes to one egg. But in Drosophila there is a stock of flies in which a certain pro-

portion of the eggs receive two X chromosomes instead of just one. This is "non-disjunctional" stock. Assume now that a female of non-disjunctional stock is hybrid at two loci in the X chromosome, thus $\dfrac{a\ b}{A\ B}$. Then if crossing over took place at the two-strand stage, two crossovers would be produced, $a\ B$ and $A\ b$. When these split to produce the tetrad, four chromosomes would be produced ($2\ a\ B$ and $2\ A\ b$) and all four would be crossovers. If non-disjunction should happen to occur and two of the chromosomes went to one egg, then *both* chromosomes would of necessity be crossovers. Assume, however, that crossing over took place at the four-strand stage. Then two of the four chromosomes of the tetrad might be crossovers ($a\ B$ and $A\ b$); the other two might be non-crossovers ($A\ B$ and $a\ b$). If non-disjunction should now take place it would be possible for a crossover and a non-crossover to go to the same egg. This we saw would be impossible if crossing over took place at the two-strand stage. The experimental evidence shows that sometimes a non-disjunctional egg contains both a crossover and a non-crossover chromosome, thus proving that crossing over takes place at the four-strand stage.

A given strand of a tetrad might undergo crossing over with either of its non-sister strands, as indicated in Fig. 76.1 left. In
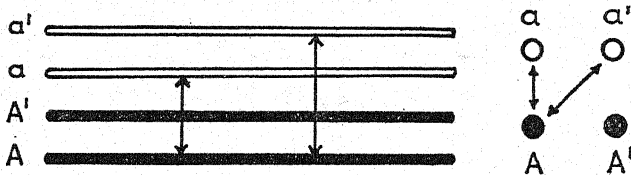
FIG. 76.1. Crossing over between non-sister strands of a tetrad. Left, a tetrad in side view; right, in end view.

this figure A and A' are sister strands (produced by the division of the same mother chromosome); so are a and a'. Either of the first two sister strands, say, A, might undergo crossing over with either of its non-sister strands (a or a'), as indicated by the arrows. The same thing is indicated in Fig. 76.1, right, but here the tetrad is represented in end view. We can refer to crossing over between A and a as *vertical*, and between A and a' as *diagonal*. Using these terms, then, crossing over occurs between non-sister strands, but it might be either vertical or diagonal.

Attached-X Stock.—In Drosophila there is a race of flies, discovered by Lilian Morgan, in which the 2 X's in the female are joined together or attached at one end, this being the end which contains the centromere, usually designated as the "right" end of the X (Fig. 76.2). Such females also contain a Y-chromosome. Hence they produce two classes of eggs: one with attached X's,
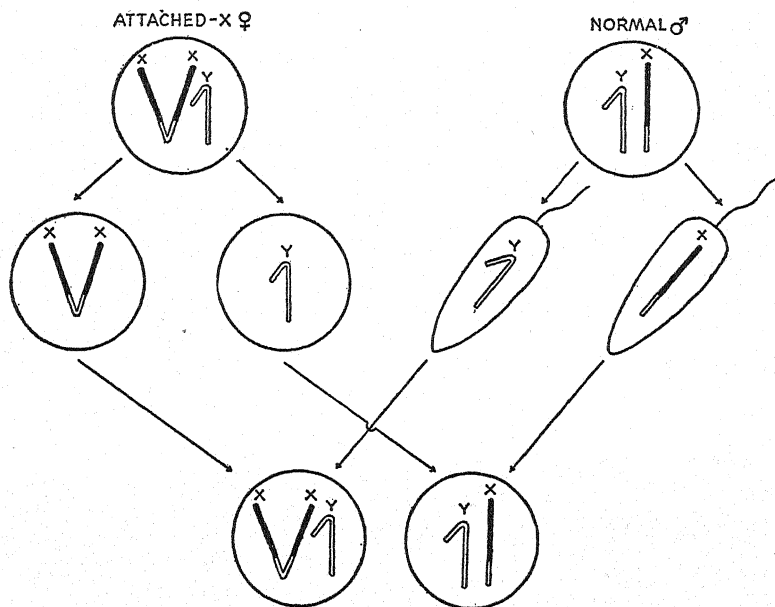
FIG. 76.2. Attached-X stock.

the other with a Y. When such a female is bred to a normal male, it produces only two classes of offspring capable of survival; namely, attached-X daughters and normal sons (Fig. 76.2). When the reduction division takes place in an attached-X female, each X first splits into two as usual, and so two pairs of attached-X's, or two "V's" are produced (Fig. 76.3). Together, these consist of four chromatids, and so they constitute a tetrad. The Y's also split into two. At the end of the reduction division the split V's separate from the split Y's, and at the equation division the split products in each case separate from each other (Fig. 76.3). Thus half the eggs receive a V and half a Y. Since two attached X's never

disjoin (separate), they always go to the same egg, and so attached-
X stock is in effect 100 per cent non-disjunctional.

Suppose an attached-X female contained, say, white (w) in one
X and the normal allele (+) in the other (Fig. 76.4, upper part).
We could represent the two X's in cross section as dots, the X's
being joined, say, below the plane of the page (Fig. 76.4, lower
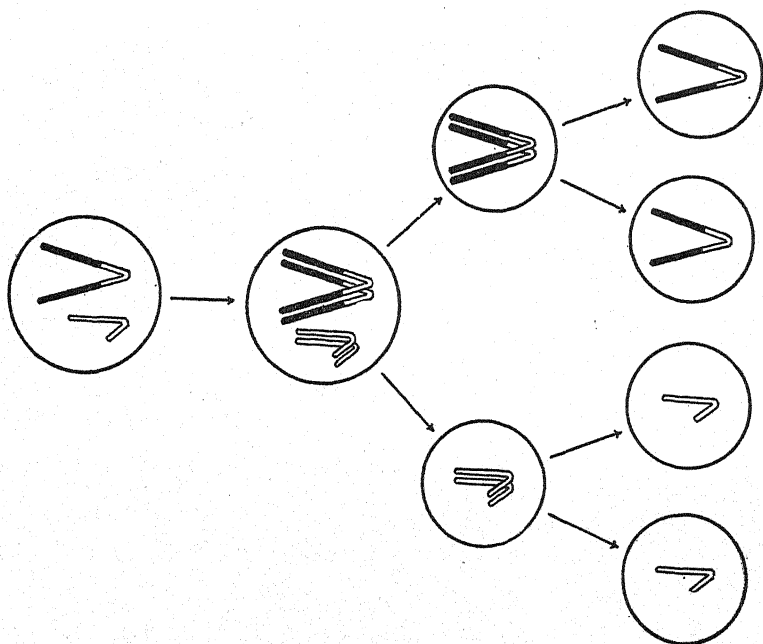
Fig. 76.3. The reduction and equation divisions in attached X-stock.

part). When the X's split they would form a tetrad containing two
w's and two +'s. The two chromatids containing either the two
w's or the two +'s would be sister chromatids (shaded alike in
Fig. 76.4). Suppose now that a diagonal crossing over took place
between the attached end of the X's and the locus of w (indicated
by an arrow in Fig. 76.4). Then the two w's would be brought
together in the same V, and the daughter which received this
would appear white-eyed. Such daughters may actually be pro-
duced, and they tell us that crossing over may be diagonal. Cross-

ing over may also be vertical. But in the present experiment vertical crossing over would simply change a V from $w/+$ to $+/w$, and therefore a more elaborate experiment is necessary to demonstrate vertical crossing over.

If crossing over took place at the two-strand stage (and only at this stage) a given V of composition $w/+$ would contain both w and $+$ after crossing over, and when it split to form a tetrad it would give rise to two V's with both w and $+$. Hence white daughters would be impossible. But we just saw that such daughters are
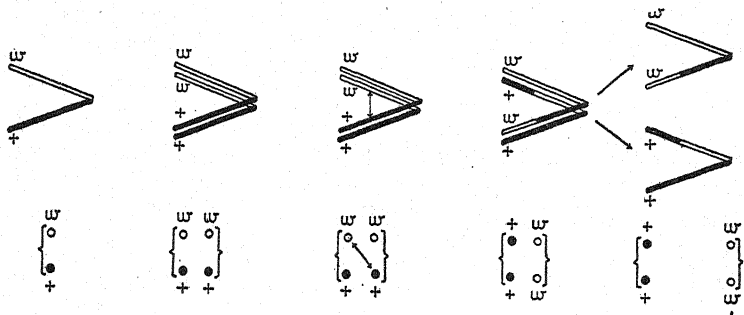
FIG. 76.4. Proof of diagonal crossing over in an attached-X female. The brackets embrace the two X's which are attached.

actually produced, and they can be produced only by diagonal crossing over at the four-strand stage. They therefore prove that crossing over takes place at this stage.

Attached-X stock has proved to be of great value in the study of problems connected with crossing over.

The Products of Multiple Exchanges.—A crossing over involves an exchange of chromosome segments and is therefore referred to as an *exchange*. The cytological evidence of an exchange is a chiasma. A single exchange results in two single crossovers. A tetrad contains four strands, and if a single exchange took place in it, only two of its four strands would be involved in crossing over. Hence the tetrad in question would yield two crossovers and two non-crossovers (Fig. 70). Two exchanges might take place in the same tetrad (Fig. 77). The second exchange (2) might involve the same two strands as the first (1), or it might involve the other two strands, or it might involve one strand in common with the first.

We shall refer to two exchanges in the same tetrad as a *double exchange*. It is evident that a double exchange might involve only two of the four strands of a tetrad (Fig. 77a) or that it might involve three (Fig. 77c), or all four (Fig. 77b). Accordingly a double exchange is referred to as a two-, three-, or four-strand exchange, respectively.

a. TWO STRAND   b. FOUR STRAND   c. THREE STRAND
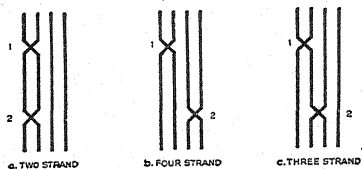
FIG. 77. Double exchanges.

Figure 78, upper left, shows a tetrad in cross section in the plane of a given exchange (X). In Fig. 78, upper right, the same tetrad is shown in section further along its length, in the plane of a second exchange. This second one might involve the same two strands as the first (a and A), and is numbered 1. The combination X-1 is a two-strand exchange. Or the second exchange might involve the other two strands (a' and A') and is numbered 4. This combination (X-4) is a four-strand exchange. Finally, the second exchange might involve either diagonal combinations 2 or 3 (one of the two strands involved in
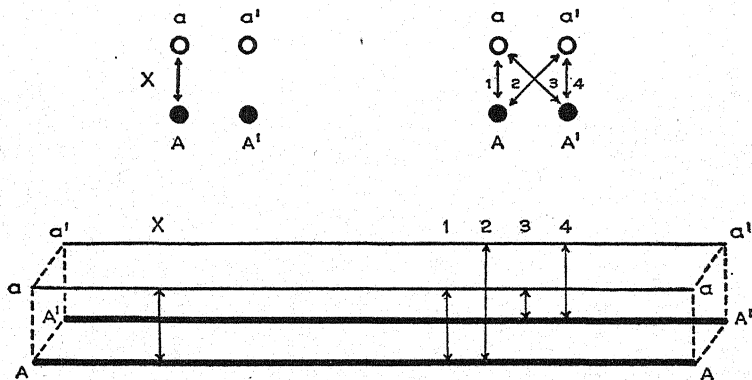
FIG. 78. The possible combinations of strands involved in a double exchange.

X and one of the other two). Each of these combinations (X-2 and X-3) represents a three-strand exchange. These combinations of X with 1, 2, 3, or 4 are shown in side view in the lower part of the figure.

In Fig. 79a, left, the combination X-1 represents a two-strand exchange. In order to get the products of this exchange we might

begin with the left end of the top strand and go to X. This gives us a light segment. At X we cross over to the dark chromosome and proceed to 1. This gives us the dark segment X-1. At 1 we cross over to the light chromosome we began with and proceed to the right end. The resulting chromosome is the double crossover
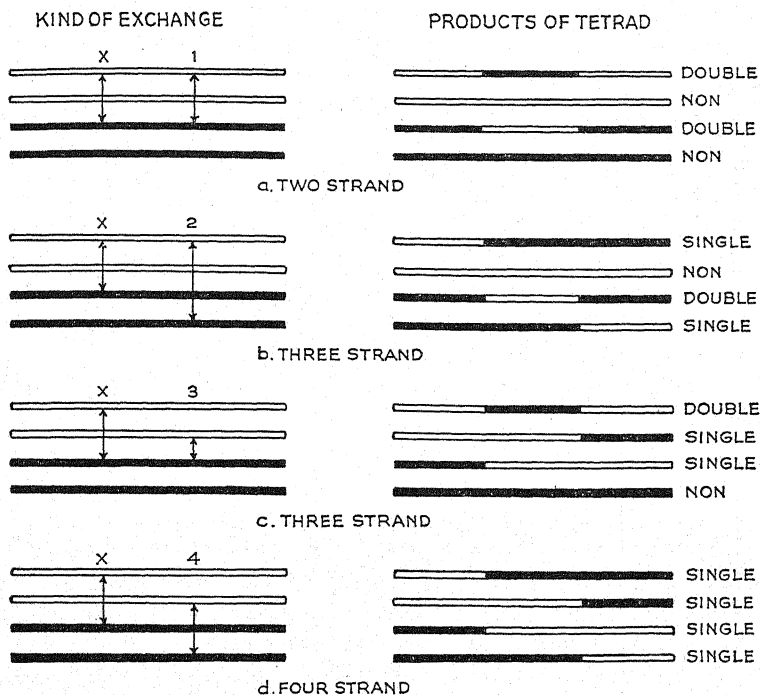
FIG. 79. The products of double exchange tetrads. The first exchange (X) involves two given strands (the same ones throughout the figure); the second exchange (1, 2, 3, 4) involves any one of four possible combinations of strands.

shown topmost in Fig. 79a, right. The second strand from the top in Fig. 79a, left, is not involved in either exchange and is a non-crossover (Fig. 79a, right, second strand from top). If we begin with the third strand from the top in Fig. 79a, left, we again get a double crossover product; and with the fourth strand, a non-crossover. Thus a two-strand exchange results in two non-crossovers and two doubles. Combination X and 2 (Fig. 79b) is a three-strand exchange; so is combination X and 3 (Fig. 79c). Each of these results in one non-crossover, two singles, and one

double crossover. The combination X and 4 (Fig. 79d) represents a four-strand exchange. It results in four single crossovers.

The above four combinations (of X and 1–4) occur with equal frequency. This is due to the fact that exchanges 1–4 occur with equal frequency, and when there is a double exchange, the first exchange (X) has no influence upon what combination of strands will be involved in the second (1–4). Hence a double exchange involves equal numbers of the four possible combinations of strands shown in Fig. 79, left. As two of these combinations involve three strands, and only one involves either two or four strands, the ratio of two-, three-, and four-strand double exchanges to one another is 1 : 2 : 1. The two-strand exchange, as above stated, produces two non-crossovers and two double crossovers. Each of the three-strand exchanges (in the above 1 : 2 : 1 ratio) produces 1 non-crossover, 2 singles, and 1 double, and the two three-strand exchanges together therefore produce 2 non-crossovers, 4 singles, and 2 doubles. The four-strand exchange produces 4 singles.

Table 4 gives in summary the relative frequencies of the different kinds of chromosomes produced by double exchanges.

TABLE 4. SUMMARY OF THE PRODUCTS OF DOUBLE EXCHANGES

Kind of Double Exchange Tetrad	Kinds of Chromosomes Produced and Their Frequencies		
	Non-cross-overs	Single Crossovers	Double Crossovers
2 strand.............................	2	..	2
3 strand.............................	2	4	2
4 strand.............................	..	4	..

The frequency of the different kinds of chromosomes produced by all kinds of double exchanges (2, 3, and 4 strand) is 4 non-crossovers : 8 singles : 4 doubles (the total got from Table 4), or 1 non-crossover : 2 singles : 1 double. Thus out of every 4 chromosomes produced by double exchange tetrads, only 1 on the average is a double crossover.

The Limit to the Per Cent of Recombinations Possible Between Two Pairs of Linked Genes.—When we were considering linkage it was stated that the new combinations between two pairs of linked genes never exceeded the old combinations. Thus suppose a hybrid were of composition $\dfrac{a\ b}{A\ B}$, where $a\ b$ are in one chromosome and $A\ B$ in the homologous chromosome. Then the new combinations would be $a\ B$ and $A\ b$, and these could never exceed the old combinations ($a\ b$ and $A\ B$). It should be borne in mind that we are now speaking of genes, not of chromosomes. The old combinations are often carried by non-crossover chromosomes but not always; they might be carried by double crossovers between a and b.

It might seem odd that when genes are linked the per cent of new combinations can never exceed the per cent of old combinations, but the reason for this becomes evident when we consider the products of any tetrad. Tetrads with one exchange produce as many non-crossovers as crossovers (two of each on the average). Hence single exchanges could never cause more than 50 per cent recombination regardless of their number. Neither could double exchanges, because double exchange tetrads produce single chromosomes in the ratio of 4 non-crossovers : 8 singles : 4 doubles (or 1 : 2 : 1) on the average, as shown in Table 4. As neither the non-crossovers nor doubles result in recombinations, and as they are equal on the average to the singles (which do result in recombinations), it is obvious that double exchanges cannot produce over 50 per cent recombinations regardless of their number. The same is true of any higher number of exchanges.

The reader must not get the impression from what has just been said that the per cent of old combinations is always equal to the per cent of new, when genes are linked. This in fact is seldom if ever true. What we have been trying to point out is that the per cent of new combinations cannot *exceed* the per cent of old. When two linked genes are very close together, there would seldom be any crossing over between them; that is to say, the limited region of the tetrad between these two genes would seldom contain an exchange. As a result most of the chromosomes produced by such tetrads would here be non-crossovers and hence most of them would contain the old combinations of genes. But when two linked genes are very far apart, say, one at each end of the chromosome,

then the region of the tetrad between the two would often contain one or more exchanges. These exchanges would produce some recombinations between the two linked genes in question and their alleles. The question then arises, could exchanges between two pairs of linked genes ever cause over 50 per cent recombination? The answer is no; regardless of the number of exchanges between two pairs of linked genes the per cent of new combinations can never exceed the per cent of old. Fifty per cent is the *limit* of recombinations possible between pairs of linked genes, as above explained.

SUMMARY

1. The pairing of the chromosomes at the reduction division is called *synapsis*. It results in (1) the reduction in chromosome number, (2) the segregation of alleles and Mendelian recombination, (3) crossing over.

2. The reduction and equation divisions are sometimes referred to as the first and second "meiotic" divisions. The changes which the chromosomes undergo during these two divisions are known as *meiosis*. These changes include (1) the pairing of the chromosomes (synapsis), (2) the splitting of the paired chromosomes into chromatids (tetrad formation), (3) crossing over, (4) the separation of homologous centromeres, each with two attached chromatids (dyad formation), and (5) the breaking up of the dyads into single chromatids (at the equation division).

3. Crossing over takes place during the tetrad stage of meiosis. A single crossing over involves only two of the four chromatids of a tetrad, and it always takes place between "non-sister" chromatids (those not produced by the splitting of the same chromosome).

4. Crossing over involves (1) the breakage of two non-sister chromatids at corresponding points and (2) the "crisscross" union of the chromatid fragments at their broken ends.

5. Crossing over results in a crossing of chromatids, referred to as a *chiasma* (pl., *chiasmata*).

6. A chiasma moves in zipper-fashion to the end of a tetrad. This is known as *terminalization*.

7. In the early embryo, before the formation of the gonads (testes or ovaries), the cells of the germ track are called "primordial germ cells." In the testes the cells of the germ tract, in the order of their succession, are (1) the spermatogonia, which cells multiply by mitosis, (2) the primary spermatocytes, which cells undergo the first meiotic division, (3) the secondary spermatocytes, which cells undergo the second meiotic division, (4) the spermatids, which cells differentiate into the sperm cells. The cor-

responding cells in the ovary are the oogonia, the primary and secondary oöcytes, the oötids, and the eggs.

8. During the prophase of the first meiotic division, the nucleus increases in size, and this stage is sometimes referred to as the "growth period."

9. The prophase of the first meiotic division (the growth period) is subdivided into (1) the *leptotene* stage (chromosomes long and thin and not yet paired), (2) *zygotene* (chromosomes in process of pairing), (3) *pachytene* (chromosomes paired but not yet split), (4) *diplotene* (the stage when the chromosomes split and form tetrads, and when chiasmata are formed as a result of crossing over), (5) *diakinesis* (the chiasmata partly terminalized and tetrads consisting of chromosomes in form of short thick rods lying close to or on the nuclear wall).

10. Like chromosomes attract one another in pairs. In mitosis the chromosomes split in the very early prophase, before they have condensed out of the chromatin. The split halves attract each other and thus the attraction of like chromosomes for each other is satisfied. A given split chromosome therefore does not attract the homologous split chromosome. In meiosis, by contrast, the chromosomes condense out of the nucleus *before* they have had time to split, and therefore homologous chromosomes attract each other and pair. In other words, homologous chromosomes pair at meiosis because they condense *precociously* out of the chromatin. This account of the cause of the pairing at meiosis is known as the *precocity theory*.

11. At meiosis homologous chromosomes, after pairing, coil about each other. This is known as *relational coiling*. It exerts a strain on the chromatids and causes breakage. The broken ends then unite in new combinations (upper "left" broken end with lower "right," and upper "right" with lower "left"). This constitutes crossing over and results in chiasma formation. The theory that chromosome breakage is caused by the strain of relational coiling might be referred to as the *strain theory* of crossing over.

12. When crossing over takes place, chromosomes do not first cross and then break (as they are often shown to do in diagrams), but they first break and then cross, as described under the strain theory. If crossing over occurred in the first way (crossing of chromosomes first), then there would be no chiasmata after breakage; actually, there are.

13. In Drosophila, Bar (*Ba*, narrow eyes) and carnation (*car*, light eyes) are in the X chromosome. It is possible to get a female of genotype $\dfrac{+ \ car}{Ba \ +}$ in which one X is visibly differentiated from another in such a way that crossing over would be expected to make them visibly different from the non-crossovers. Among the offspring of such a female, those which are genetic crossovers are found, upon microscopic examination, to contain the

crossover chromosomes and those which are not genetic crossover do not contain the crossover chromosomes. This constitutes "cytological" proof of crossing over.

14. In meiosis the chromosomes at first pair and then split (forming a tetrad), and accordingly a pair at first consists of two "strands" and then of four. The two corresponding stages in meiosis are sometimes called the "two-strand" and the "four-strand" stages.

15. In Drosophila, an egg sometimes gets two X's (in "non-disjunctional" stock), one of which is a non-crossover, the other a crossover. This proves that crossing over takes place at the four-strand stage, for only then might a given tetrad contain both crossovers and non-crossovers (two of the four strands having been involved in crossing over, and two not). If now two X's happened to go to the same egg (as in "non-disjunctional" stock), then one might be a crossover and the other a non-crossover.

16. In Drosophila, the females of "attached-X" stock contain two attached X's and a Y chromosome. They produce eggs of two classes: (1) attached-X and (2) Y. The union of an attached-X egg and a Y-containing sperm cell (from a normal male) produces an attached-X daughter.

17. In an attached-X female, each X splits at the reduction division, producing a tetrad, consisting of two pairs of attached X's (or two V's).

18. If an attached-X female contains white (w) in one X and the normal allele ($+$) in the other, then the tetrad (formed by the splitting of the V) would contain 2 V's, each with a w and a $+$. A "diagonal" crossover between w and the centromere might produce a V pure for w. A daughter which received such a V would be white-eyed and would tell us that diagonal crossing over had taken place. Crossing over might also be "vertical," but a more elaborate experiment is necessary to show this (one again involving the use of attached X's).

19. The recombination of chromosome segments involved in crossing over is sometimes referred to as an *exchange*.

20. A single exchange involves only two of the four chromatids of a tetrad, and therefore a single exchange tetrad produces two crossovers and two non-crossovers. Therefore single exchanges cannot result in more than 50 per cent recombination between gene pairs. It can also be shown that double exchanges cannot result in more than 50 per cent recombination between gene pairs, since both exchanges would often involve the same two chromatids, or only three of the four chromatids of a tetrad (as when one exchange is "vertical" and the other "diagonal"); and in such cases the exchanges would produce either no recombination (as when both exchanges involve the same two chromatids) or as many old combinations as new (as when the two exchanges involve only three chromatids). Neither can triple exchanges, nor any higher order of exchange, result in more new recombinations than old. Hence, there cannot be more than 50 per cent recombination between pairs of linked genes.

PROBLEMS

1. Given 100 tetrads in each of which there is one chiasma. Tell (1) how many chromatids (or chromosomes) the 100 tetrads would produce and (2) how many of these chromatids would be crossovers. Therefore, what per cent of the original tetrads contain a chiasma (if each of the original 100 contained a chiasma), and what per cent of the resulting chromatids contain crossovers (that is, how many crossovers are there per hundred *chromatids*)? In general, how does the per cent of chiasmata (that is, number of chiasmata per 100 *tetrads*) compare with the per cent of crossovers (number of crossovers per 100 *chromatids*)?

2. If the number of chiasmata per tetrad was four (or 400 per cent chiasmata) for a chromosome of a certain length, what would be the per cent of crossovers over the entire length of the chromosome? Therefore, what would be the length of the chromosome?

3. Suppose that two genes, a and b, are 20 units apart in a chromosome. What is the per cent of crossing over between the two (or number of chromatids per 100 that are crossovers between a and b)? What is the per cent of chiasmata between a and b (that is, per cent of tetrads with chiasmata between a and b)?

Note. Problems 4–12 constitute a series dealing with the proof that crossing over might be either vertical or diagonal.

4. Assume that in Drosophila a female with attached X's contains white eyes (w) in one X and the normal allele ($+$) in the other. Let us indicate the attached X's in cross section as $\overset{w}{\underset{w}{\circ}}$ (the two X's being connected, say, below the plane of the page), and let us indicate a tetrad (formed by the splitting of each X at the reduction division) as $\overset{w\ w}{\underset{+\,+}{\circ\ \circ}}$. (Sister chromatids are shaded alike.) If this female is mated to any male, what will be the genotype and phenotype of her daughters, assuming that there is no crossing over?
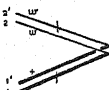
5. Assume that in the above attached-X female $\left(\overset{w\ w}{\underset{+\,+}{\circ\ \circ}} \right)$ a "diagonal" exchange takes place, say, between the lower left ($+$) and the upper right (w) chromatid, and that this exchange takes place in the interval between the attached ends of the X's (the centromere) and the locus of w. Give the composition of each pair of attached X's (formed by diagonal crossing over).

6. Indicate the above attached-X tetrad $\begin{pmatrix} w & w \\ \circ & \circ \\ \bullet & \bullet \\ + & + \end{pmatrix}$ in longitudinal view

thus: . Let one V (1·2) be in the plane of the page, the other

one (1′·2′) being below the plane of the page; and let us designate the first (1·2) as the "near" V, the second (1′·2′) as the "far" V, and the chromatids nearer the top of the page as "upper," those nearer the bottom as "lower." Assume that a diagonal exchange takes place between chromatids 1 and 2′ (lower near and upper far) at the points indicated by the short cross lines. Make separate drawings of the resulting two V's. Give next the genotypes and phenotypes of the daughters which receive the V's in question (resulting from a diagonal exchange). Tell which of these daughters would tell you that diagonal crossing over had occurred in the mother.

7. Given an attached-X tetrad of constitution $\begin{matrix} w & w \\ \circ & \circ \\ \bullet & \bullet \\ + & + \end{matrix}$. Assume that a vertical exchange takes place, say, between the lower left chromatid ($+$) and the upper left (w), in the interval of the chromosome between the centromere and the locus of w. Then tell what the composition of the resulting V's will be and give the appearance of the daughters that receive the attached X's in question. Will they appear any different from the non-crossovers? Would you say then that an attached-X female as simple as $w/+$ could inform you of vertical crossing over?
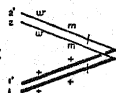
8. Given the following attached-X tetrad: . Assume that a diagonal exchange takes place between chromatids 1 and 2′ (lower near and upper far) at the points indicated by the short cross lines. Make separate drawings of the resulting two V's and give the genotypes and phenotypes of the daughters that receive them (m standing for miniature wings, recessive to $+$ for long wings).

9. Given the following attached-X tetrad . Assume that a diagonal exchange takes place between chromatid 1 and 2′ (lower near and upper far) between m and the centromere (as indicated by the short cross lines). Give the constitution of the resulting "near" V; give also that of the resulting "far" V. What would be the appearance of the daughters that received these V's?

10. In the above attached-X tetrad would an exchange ever result in a V of constitution $\dfrac{w\,m}{w\,m}$? Suppose then that an attached-X female was of genotype $\dfrac{+\,m}{w\,+}$ (from which the tetrad in Problem 9 is derived). Could such a female give rise to white miniature $(w\,m)$ daughters? Would attached-X females of constitution $\dfrac{w\,m}{+\,+}$ and $\dfrac{+\,m}{w\,+}$ be any different in appearance? Tell, then, how you could distinguish between them (by a difference in the kinds of daughters they produce).

11 Given an attached-X female of constitution $\dfrac{w\,m}{+\,+}$. Give the constitution of the non-crossover V's and of the vertical crossovers between w and m, and tell how you would distinguish between the daughters that receive the two kinds of V's.

12. Crossing over might be either "diagonal" or "vertical." Tell how you could show that it might be diagonal (with the aid of attached-X females of genotype $w/+$); or, that it might be vertical (with the aid of attached-X females of genotype $\left.\dfrac{w\,m}{+\,+}\right)$.

13. Starting with an attached-X female of genotype , show
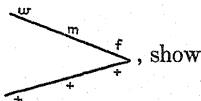
diagrammatically what kind of an exchange would be necessary (after tetrad formation) to produce daughters of the following genotypes.
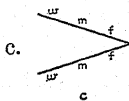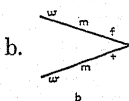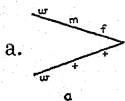
12. *MULTIPLE ALLELES*

SOMETIMES a given kind of gene mutates in two different ways. Consequently the original gene and the two mutated are all at the same locus and are alleles. When there are more than two kinds of alleles for a given locus we speak of them as *multiple alleles*. In the absence of mutation there is just one kind of allele at a given locus, namely, the normal allele. A mutation results in the addition of a second kind, and as there are just two we do not refer to them by any particular term indicative of their number. It is only when there are three or more kinds of alleles for a given locus that we refer to them as multiple alleles. We might then define multiple alleles as the members of a series of three or more kinds of genes at a given locus.

An Example of Multiple Alleles.—To give an example of multiple alleles. In Drosophila the wings are normally long. Two mutations took place at the same locus (but in different flies), one causing vestigial wings, the other "antlered" wings (Fig. 80). Since both vestigial and antlered arose in the same locus, they are both alleles of the same normal gene and also of each other. Both are recessive to their normal allele, as shown when each race is crossed to normal (the F_1 being normal in both cases). Vestigial was found first and given the symbol vg, and the locus it occupies was named after it in accordance with the procedure for naming loci. Antlered was given the symbol vg^a, the base letters vg indicating that the mutation occurred at the vestigial locus, and the superscript a that the mutation was to antlered. Using these symbols for the mutated genes and $+$ for their normal allele, we have the following genotypes for the three races: $+/+$ (long), vg/vg (vestigial), and vg^a/vg^a (antlered). Figure 80 gives chromosome diagrams of the three races, only the chromosome relevant to

the discussion being shown. The F_1 produced by a cross of long by vestigial ($+/+ \times vg/vg$) is $vg/+$. The F_2 is 1 $+/+$ (long) : 2 $vg/+$ (long) : 1 vg/vg (vestigial), or 3 long : 1 vestigial. The F_1 produced by a cross of long by antlered ($+/+ \times vg^a/vg^a$) is $vg^a/+$ (long). The F_2 is 1 $+/+$ (long) : 2 $vg^a/+$ (long) : 1 vg^a/vg^a (antlered), or 3 long : 1 antlered.

LONG WINGS
(NORMAL)

VESTIGIAL
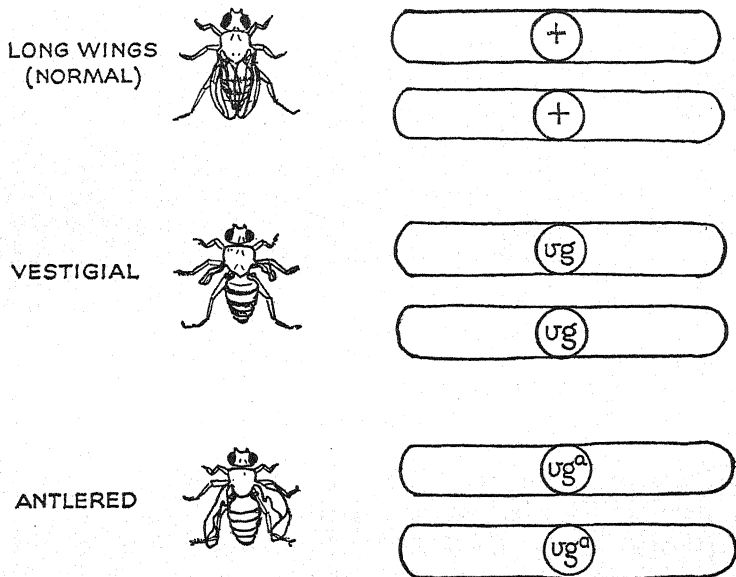
ANTLERED

FIG. 80. A case of multiple alleles in Drosophila.

When vestigial is crossed to antlered ($vg/vg \times vg^a/vg^a$) the F_1 is vg^a/vg. This fly is intermediate in wing length between vestigial and antlered. Thus neither mutated gene is either dominant or recessive. The hybrid vg^a/vg is sometimes referred to as the vestigial-antlered *compound*, from the fact that it contains two mutated genes at the same locus. When the compound flies are inbred ($vg^a/vg \times vg^a/vg$), they produce offspring in the ratio of 1 vg/vg (vestigial) : 2 vg^a/vg (compounds, intermediate) : 1 vg^a/vg^a (antlered).

Vestigial and antlered are not the only mutations at the vestigial locus. There are at this locus over a dozen additional mutations, known as strap, no wing, nicked, and so forth.

Distinguishing Between Mutations at Different Loci and at the Same Locus.—Suppose now that vestigial and antlered were not at the same locus but at different loci. Then we should give antlered a separate symbol from vg, say, a. Thus we should label the two mutations vg and a, and we should indicate the normal allele of each by a separate $+$ sign. The gametes of the three races would be $+$ $+$ (long), vg $+$ (vestigial) and $+$ a (antlered). If the two mutant loci were in separate chromosomes, then a cross of vestigial by antlered would produce F_1 of genotype $\dfrac{vg\ +}{+\ a}$.

If vg and a were at different loci in the same chromosome pair, then the F_1 would be $\dfrac{vg\ +}{+\ a}$ (the same as before except that the horizontal line is continuous to show that vg and a are in the same chromosome pair). In either event, when vestigial was crossed to antlered the F_1 produced $\left(\dfrac{vg\ +}{+\ a}\ \text{or}\ \dfrac{vg\ +}{+\ a}\right)$ would contain both normal alleles ($+$ and $+$); and since the normal alleles are dominant, the F_1 would appear normal. Actually they are intermediate between vestigial and antlered, and so they do not contain the normal allele of either mutant gene.

There would also be a difference in the F_2 results when the F_1 were inbred, if vg and a were at different loci instead of at the same locus. For, if vg and a were in separate chromosomes and the F_1 were $\dfrac{vg\ +}{+\ a}$, then by Mendelian recombination they would form four classes of gametes, one of which would be $+$ $+$ and another vg a. Therefore when the F_1 interbred, they would form some F_2 offspring of class $\dfrac{+\ +}{+\ +}$ and some of class $\dfrac{vg\ a}{vg\ a}$. The first would be pure longs, the second pure vestigial antlered. Actually, neither of these recombination classes is formed. The only F_2 classes that can be got by crossing vestigial and antlered are pure vestigial (vg/vg), compounds (vg/vg^a), and pure antlered (vg^a/vg^a).

If vestigial and antlered were at different loci in the same chromosome pair, but not too close together, then crossing over could take place between them, and again the hybrid $\left(\dfrac{vg\ +}{+\ a}\right)$ would form some gametes of class $+$ $+$, and some of class $vg\ a$. By

means of further matings it would be possible to get pure normals $\left(\dfrac{+\ +}{+\ +}\right)$ and pure vestigial antlered $\left(\dfrac{vg\ a}{vg\ a}\right)$. These would be the crossover classes, but actually no crossover classes are formed.

If vg and a were very close together in the same chromosome, then there would be no crossing over between them in the hybrid $\left(\dfrac{vg\ +}{+\ a}\right)$. Therefore the hybrid would form only two classes of gametes, namely, $vg\ +$ and $+\ a$ (the non-crossover classes). In this case it would not be possible by further matings to get the crossover classes $\left(\dfrac{+\ +}{+\ +}\ \text{and}\ \dfrac{vg\ a}{vg\ a}\right)$. Thus the absence of the crossovers would not necessarily prove that the two mutations were at the same locus. We should therefore be dependent upon the appearance of the F_1 (which are not $\dfrac{vg\ +}{+\ a}$ but are vg/vg^a, and therefore do not appear normal).

Other Cases of Multiple Allelism.—Cases of multiple allelism are by no means rare. In fact, it seems to be the rule rather than the exception for a gene to mutate in more than one way. An outstanding case of multiple allelism is one concerned with eye color in Drosophila. It will be recalled that the normal eye color of Drosophila is red and that a mutation in the X chromosome changed the normal red eye color to white. Over a dozen other mutations have occurred at the same locus, and they have changed the eye color to various lighter shades of red, known as cherry, blood, eosin, ivory, cream, etc. The mutant genes have received corresponding names and symbols, as for example eosin (w^e), the letter w telling us that the mutation is at the white locus and the superscript e that the eye color is eosin. When eosin and white are crossed, the daughters receive an X from each parent and are eosin-white compounds, w^e/w. These are intermediate between eosin and white in eye color. A cross of eosin and apricot gives the compound w^e/w^a, again intermediate in eye color. In general, when any two of the mutants in the series are crossed the F_1 are intermediate.

In rabbits there is a series of multiple alleles concerned with coat color. Rabbits normally have a uniform brown coat. The mutant races include albino and "Himalayan," a race that some-

what resembles an albino but has dark extremities (dark feet, nose, ears and tail). The mutant genes albino (a) and Himalayan (a^h) occupy the same locus (are alleles) and both are recessive to their normal allele ($+$). Albino $\times$ Himalayan ($a/a \times a^h/a^h$) gives the F_1 compound a^h/a. This is not intermediate as is usual for compounds but happens to be Himalayan. When the F_1 Himalayan are inbred ($a^h/a \times a^h/a$), they produce offspring in the ratio of 1 a^h/a^h (Himalayan) : 2 a^h/a (Himalayan) : 1 a/a (albino).

In mice there are at least two series of multiple alleles concerned with coat color. One series is at the albino locus (Fig. 81, left).
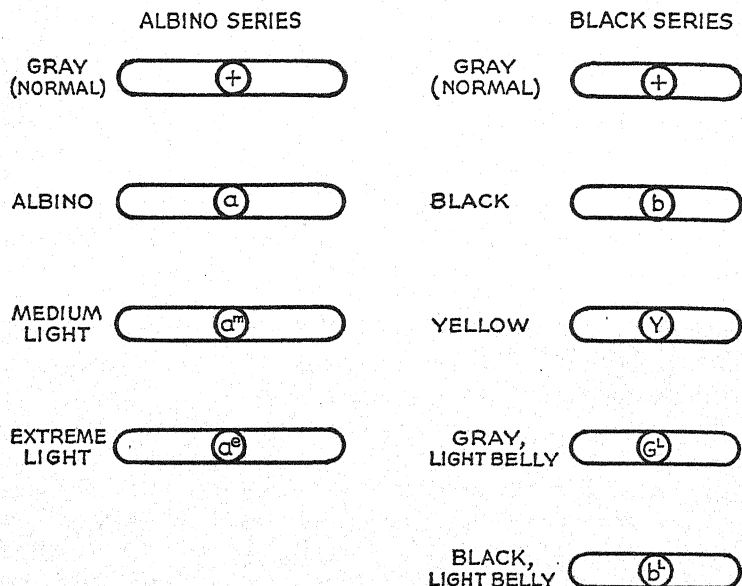
FIG. 81. Multiple alleles in mice.

The normal allele in this series is one of the numerous genes necessary for gray coat color and can be indicated by a $+$ sign. In addition, the series contains the mutant genes albino (a), medium light gray (a^m), and extreme light (a^e). All three mutant genes are recessive to the normal allele, and the compounds of any two mutants are intermediate. A second series of alleles is at the black locus and is in a different chromosome from the first series

(Fig. 81, right). In the second series the normal allele $(+)$ again is one of the numerous genes necessary for gray coat color, but of course it is a different normal from the one in the first series. In addition the second series contains the mutant genes black (b), yellow (Y), gray body with light belly (G^L), and black body with light belly (b^L). Black is recessive to all other alleles in the series and yellow is dominant to all. The light belly effect of both G^L and b^L is dominant to the darker belly of the normal gray $(+)$, but the black body effect of b^L is recessive to gray. Thus $G^L/+$ is gray light belly; so is $b^L/+$.

In corn there is a series of multiple alleles which influence seed color. The normal seed color in corn is purple and is due to purple pigment in a layer of cells just below the seed coat (the "aleurone" layer). A recessive mutation changed the purple aleurone to albino; another recessive in the same locus changed it to light purple. The corresponding mutated genes can be designated as a and a^l, and the normal gene from which they arose as $+$. The series in corn then is $+$ (purple aleurone), a (albino), a^l (light purple aleurone). The mutations a and a^l influence other parts of the plant besides the aleurone in ways that we need not consider for our present purposes.

Multiple Alleles in Relation to Self-sterility.—Multiple alleles are concerned with self-sterility in plants. This fact was first worked out in tobacco plants by E. M. East. Some strains of tobacco are self-sterile in that they fail to set seeds when they are self-pollinated. The self-sterility is due to a series of alleles designated as S_1, S_2, S_3, etc. A plant might be hybrid for any two alleles, but it cannot be pure for any one. Thus a plant might be S_1/S_2, or S_1/S_3, or S_2/S_3, etc. Now pollen containing either kind of allele present in a given plant cannot grow down the pistil of that plant. Thus, if we consider plant S_1/S_2, pollen carrying either S_1 or S_2 cannot grow down its pistils. Thus the plant is self-sterile. Moreover even if pollen carrying S_1 or S_2 comes from another plant it cannot grow down the pistils of S_1/S_2. But if some pollen from another plant carried an allele other than S_1 or S_2, say, S_3, then this could grow down the pistils of S_1/S_2, and the offspring would be S_1/S_3 and S_2/S_3. Thus automatically every plant is hybrid.

Self-sterility is found not only in tobacco but in many other

flowering plants, and it often depends on multiple alleles. The value of self-sterility to the species is that it causes a certain amount of outbreeding.

The Human Blood Groups.—Multiple alleles are concerned with differences in the immunity reactions of the blood. This was discovered in human beings by Landsteiner. We shall first describe the nature of the immunity reactions before giving their genetic basis.

It is a well-recognized fact that when blood transfusions are made, the red blood corpuscles of one person might be destroyed in the blood stream of another person, unless the proper precautions are taken. The reason for this is that some people's corpuscles contain a substance which makes their corpuscles susceptible to destruction by the blood of certain other people. The substance within the corpuscles is known as an *anti-gen*. The blood which destroys the corpuscles in turn contains a substance known as an *anti-body*. This substance combines with the substance within the corpuscles (the anti-gen). It thereby makes the corpuscles stick together in clusters and it eventually destroys them. The sticking together of the corpuscles is known as *agglutination*, and the anti-body involved is known as an *agglutinin*. This anti-body is contained in the fluid part of the blood (the plasma), and can be got in the serum (the blood minus its corpuscles and minus the proteins that cause it to clot). We can therefore conveniently say that the *serum* contains the agglutinin.

There are two kinds of anti-gens and two corresponding agglutinins. We can designate the anti-gens as A and B and the agglutinins as a and b. Thus blood corpuscles containing anti-gen A are agglutinated by blood serum containing agglutinin a; blood corpuscles with B by agglutinin b. A given person would of course not have an agglutinin against his own corpuscles, for if he had his own corpuscles would be destroyed.

Any two people who have the same kind of anti-gens in their corpuscles (or the same kind of agglutinins in their serum) are regarded as belonging to the same *blood group*. In general the human race can be divided into four blood groups. These have received various designations, but for our present purposes we can number them I, II, III, IV and in addition designate them by the symbols ab, Ab, aB, AB. Put in tabulated form, with corre-

sponding groups on the same line, the human blood groups would
then be:

<div align="center">

Blood Groups

I	*ab*
II	*Ab*
III	*aB*
IV	*AB*

</div>

The serum of one group may or may not destroy the corpuscles of
another group, depending on the group in each instance. In general,
the small letters represent agglutinins and these destroy the cor-
puscles of any group which contains anti-gens represented by the
corresponding large letters. Thus the serum of group *ab* (the first
group above) destroys the corpuscles of the three remaining groups
(*Ab, aB, AB*), since each of these contains either *A* or *B*, or both
A and *B*. But since persons in group *ab* contain neither *A* nor *B*,
their corpuscles cannot be destroyed by the blood of any of the
other three groups. Persons in group *Ab* (the second group) con-
tain *b*, and hence their serum destroys the corpuscles of groups
with *B*, namely, *aB* and *AB* (third and fourth groups). The cor-
puscles of group *Ab*, however, contain *A* and hence are destroyed
by the serum of the two groups which contain *a*, namely, *ab*
(first group) and *aB* (third group). And likewise for the remaining
two groups. Briefly stated, "small letters" agglutinate correspond-
ing "large letters," but large do not agglutinate small.

In designating the blood groups we might simply use the large
letters and omit the small, thus: *O, A, B, AB*. This is the Inter-
national nomenclature and is the one usually employed. Table 5
gives the reaction of each group with the others, and the percentage
of people in a white population belonging to each group.

<div align="center">

TABLE 5

</div>

Blood Group	Agglutinates Corpuscles of	Agglutinated by Serum of	Per Cent in Population
O................	A, B, AB	None	37.3
A................	B, AB	O, B	43.7
B................	A, AB	O, A	13.4
AB...............	None	O, A, B	5.7

When a blood transfusion is made, it does no harm if the *donor's*
blood contains anti-bodies against the recipient's, for the donor's

blood is small in amount compared to the total volume of the
recipient's; and therefore the anti-bodies are diluted. But it would
do harm if the *recipient's* blood had the anti-bodies, since now
the amount of anti-body is relatively large. A person of blood
group O for example could not be a recipient of blood from any
other group but his own, but he might be a donor for any group.

The Genetic Constitution of the Human Blood Groups.—
We do not know which of the four blood groups is the normal one.
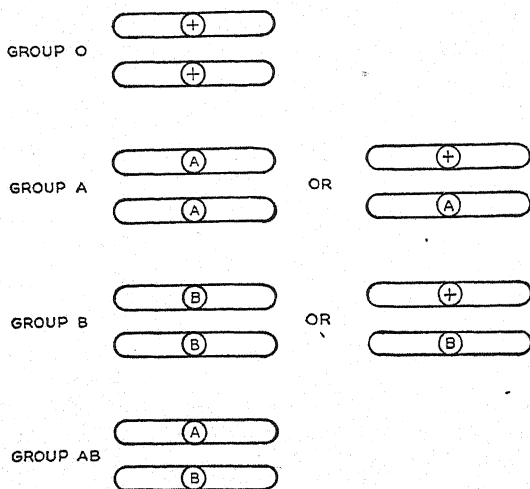
FIG. 82. Chromosome diagrams of the human blood groups (unreduced cells).

Ordinarily individuals with normal traits are the most numerous
in any species. Both groups O and A are fairly numerous (37.3 and
43.7 per cent of the total, respectively) and either might be con-
sidered the normal. For convenience we shall regard class O as
the normal. Then groups A and B arose from group O as the result
of two dominant mutations (one for each group). The mutant
genes can be given the symbols A and B, respectively. Both of
these arose in the same locus from one of the normal genes in
class O. We can designate the normal gene in question by the
symbol $+$. Thus the three genes $+$, A, and B occupy the same
locus and are multiple alleles. Since $+$ is a recessive, group O must
be pure for $+$; that is, its formula is $+/+$ (Fig. 82). Since gene A
is a dominant, group A might be either pure (A/A) or it might be

hybrid $(+/A)$. In like manner, group B might be either B/B or $+/B$. Group AB on the other hand is *always* hybrid and is of composition A/B.

It will be seen that if both parents in a given family are of class O, they must both be of genotype $+/+$, and all of the children must therefore also be $+/+$; that is, of class O, like their parents. If, on the other hand, both parents were of class A but both happened to be hybrid $(A/+)$, then they might have some children of class O in addition to some of their own class, since $A/+ \times A/+$ gives offspring in the ratio of $1\ A/A : 2\ A/+ : 1\ +/+$. However, if both parents were of group A, then the children would have to belong to either group A or group O, and we could affirm that a child belonging to any other group was not the offspring of a man of group A if the mother were also of group A. In cases where the paternity of a child is in question, therefore, blood tests could be used as evidence only under restrictions of the kind just indicated.

Group A/B is rather unusual in that it shows the full effect of both alleles (A and B). Usually a hybrid that has two mutant alleles at the same locus is intermediate between the two mutants, as for example vestigial-antlered or eosin-white in Drosophila. But in some cases of multiple allelism, the hybrid does show the full effect of both mutant alleles.

The Human Blood Groups and Mutation.—The human blood groups are rather remarkable in point of origin. The mutant groups A and B not only contain the anti-gen that makes their corpuscles susceptible to agglutination by the normal serum (of group O), but they also lack the agglutinins present in the normal serum. Therefore, each mutation must have done two things: (1) it must have resulted in the production of a substance (an anti-gen) that made the corpuscles susceptible to agglutination (by an agglutinin) and (2) it must have resulted in the dropping out of the agglutinin (the anti-body). It is indeed remarkable that a mutation should have resulted not only in the production of an anti-gen but also in the dropping out of an anti-body against *that very anti-gen*, and that this sort of thing should have happened not just once, but *twice*, once in the production of blood group A and again in the production of blood group B. The further study of these facts might shed some light on the nature of immunity and of genes themselves.

SUMMARY

1. A normal diploid cell does not contain more than two alleles at a given locus, since it contains only one pair of chromosomes of each kind. But a given locus might be occupied by several different kinds of alleles in several different individuals, and the alleles are then referred to as *multiple alleles*.

2. In Drosophila, the wings are normally long. Vestigial (*vg*, wings reduced to stumps) was the first mutant gene discovered at its locus, and the locus was named after it. Several additional mutations occurred at this locus, including "antlered" (wings intermediate between vestigial and long). Antlered was labeled vg^a, "*vg*" indicating that it was at the vestigial locus and "*a*" that it was antlered.

3. Both vestigial and antlered are recessive to their normal allele (+, for long wings), and when they are crossed to long (+/+ × *vg/vg*, or +/+ × vg^a/vg^a), the F_1 are long (*vg*/+ or vg^a/+) and the F_2 are 3 long : 1 mutant (1 +/+ : 2 *vg*/+ : 1 *vg/vg*, or 1 +/+ : 2 vg^a/+ : 1 vg^a/vg^a).

4. When vestigial is crossed to antlered, the F_1 are vg/vg^a. They appear intermediate between vestigial and antlered and are referred to as the vestigial-antlered "compound." The F_1 when interbred produce F_2 in the ratio of 1 *vg/vg* (vestigial) : 2 vg/vg^a (intermediate) : 1 vg^a/vg^a (antlered).

5. If antlered were not at the same locus as vestigial, then vestigial gametes would be *vg* + and an antlered + *a* ("*a*" now standing for antlered), and a cross of the two would produce F_1 of genotype $\dfrac{vg\ +}{+\ a}$ (if *vg* and *a* were in separate pairs of chromosomes) or $\dfrac{vg\ +}{+\ a}$ (if they were in the same pair). The F_1 would therefore be normal, since each normal allele would be dominant to its mutant allele. The fact that the F_1 are intermediate between vestigial and antlered indicates that they do not contain the normal allele of either mutant gene, and that they are vg/vg^a.

6. If the F_1 from vestigial × antlered were $\dfrac{vg\ +}{+\ a}$ (vestigial and antlered in separate chromosome pairs), then by Mendelian recombination they would produce some gametes of composition + + and *vg a*. The first (+ +) would produce normals in the F_2 and the second (*vg a*) would produce some pure vestigial antlered $\left(\dfrac{vg\ a}{vg\ a}\right)$. Actually, the F_2 contains neither normals (+ +) nor pure vestigial antlered $\left(\dfrac{vg\ a}{vg\ a}\right)$. Moreover, if the F_1 were of genotype $\dfrac{vg\ +}{+\ a}$ (instead of vg/vg^a), they would produce F_2

in the ratio of $9 + +$ (normal) : $3\ vg +$ (vestigial) : $3 + a$ (antlered) : $1\ vg\ a$ (vestigial antlered), instead of $1\ vg/vg$ (vestigial) : $2\ vg/vg^a$ (intermediate) : $1\ vg^a/vg^a$ (antlered).

7. If vestigial and antlered were in the same chromosome pair but not too close together, then by crossing over an F_1 female of genotype $\dfrac{vg +}{+\ a}$ would produce some eggs of composition $+ +$ and $vg\ a$, and in a later generation it would be possible to derive, from such an F_1 female, some normals $(+ +)$ and some pure vestigial antlered $\left(\dfrac{vg\ a}{vg\ a}\right)$. But this is not possible.

8. If vestigial and antlered were in the same chromosome pair but so close together that there was no crossing over between them, then the F_1 female $\left(\dfrac{vg +}{+\ a}\right)$ would not produce the crossover classes $+ +$ and $vg\ a$, and in effect $vg +$ would act like a single gene and $+ a$ like its allele. In a case of this sort, it would be difficult to tell whether we were dealing with a single pair of alleles or two pairs. But the F_1 appear intermediate between vestigial and antlered and this indicates that they are vg/vg^a rather than $\dfrac{vg +}{+\ a}$. The appearance of the hybrid is then the only means of distinguishing between cases of multiple allelism and complete linkage.

9. In Drosophila cases of multiple allelism are the rule rather than the exception.

10. An outstanding example of multiple allelism in Drosophila is the series of alleles at the white-eye locus consisting of the normal allele of white and over a dozen mutant genes, each causing eye colors lighter than the normal (red), ranging from only a slight shade lighter than red to white and known as cherry, blood, eosin, tinged, ivory, etc. Each mutant gene is given the base symbol "w" (the locus having been named after white, the first mutant gene discovered at the locus in question). Eosin, for example, is w^e. All the mutant members of the series are recessive to the normal allele ($+$, for red), but the compounds (containing any two different mutant genes) are in all cases intermediate between the pure mutant types.

11. Cases of multiple allelism are common both among plants and animals. In rabbits, albino (a), Himalayan (a^h), and their normal allele ($+$, for brown coat) form a series. In mice, both the albino and black loci contain a series of multiple alleles. In corn, there is a series which influences the color of the aleurone and other parts of the plant, and consists of dark purple $(+)$, albino (a), and light purple aleurone (a^l).

12. In tobacco sterility is dependent on a series of multiple alleles $(S_1,\ S_2,\ S_3,$ etc.). For example, a plant of genotype S_1/S_2 cannot be pol-

linated by pollen containing either S_1 or S_2. Hence it is self-sterile. The advantage of self-sterility to the plant is that it makes cross-pollination necessary and thereby leads to hybrid vigor. It is probable that sterility is often caused in plants by multiple alleles, as just described for tobacco.

13. The human blood groups are determined by multiple alleles. If we regard group O as normal $(+)$, then groups A and B arose as the result of two dominant mutations, A and B, the series of alleles thus being $+$, A and B. Group O is $+/+$, group A is either A/A or $+/A$; group B is either B/B or $+/B$, and group AB is A/B.

PROBLEMS

1. In mice, four members of the series of alleles at the black locus, in the order of dominance, are: gray light belly (G^L), gray $(+)$, black light belly (b^L) and black (b); but the light belly effect of b^L is dominant to the non-light belly of the normal gray mouse $(+)$. Give the appearance of the parents in the following crosses, and the genotypes and appearance of their offspring.

a. $G^L/+ \times b/b$	**c.** $G^L/+ \times b^L/b$
b. $b^L/+ \times b/b$	**d.** $b^L/+ \times b^L/b$

2. Give four possible genotypes for a heterozygous gray light belly mouse. Tell which combinations of these possible genotypes would produce gray light belly and black light belly offspring, but no others. Include parents of the same genotype among the possible combinations.

3. To what kind of a mouse might any gray light belly be bred in order to determine its genotype?

4. Give the possible blood groups of the offspring if both parents are of group (1) A, (2) B, (3) AB, (4) O.

5. Give the possible blood groups of the offspring when the parents are of the following blood groups: (1) $A \times B$, (2) $A \times AB$, (3) $A \times O$, (4) $B \times AB$, (5) $B \times O$.

6. Give all the possible blood groups to which the father of a child might belong (1) if the mother belongs to A and the child to group B, (2) if both mother and child belong to group O.

7. According to the "presence and absence theory" the only possible alleles of a given gene are either itself or its "absence" (mutants always arising by the loss of a gene). Are cases of multiple allelism evidence for or against this theory?

8. Assume that races A and B give a 3 : 1 ratio in the F_2 when crossed and that races A and C also do. Would B and C necessarily give a 3 : 1 ratio? Tell why or why not.

13. *ABNORMAL CHROMOSOMAL REARRANGEMENTS*

C̲HROMOSOMES might be broken up into segments, and the segments might become reattached in new arrangements. This happens in an orderly manner when crossing over takes place. But occasionally chromosome segments undergo irregular breakage and rearrangement. We shall now consider cases of this sort.

Translocations.—In Fig. 83, left, two non-homologous chromosomes are shown above and each is arbitrarily considered as
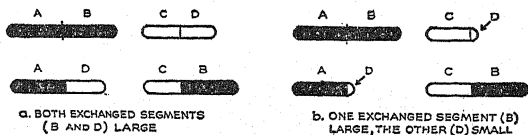
FIG. 83. Translocations (or segmental interchanges).

made up of two segments, labeled AB and CD respectively. By means of X-rays it would be possible to break up each chromosome accidentally into the two segments in question. Next A and D might become attached and also C and B. This would give rise to AD and BC, shown in Fig. 83, left. We might think of AB and CD as giving rise to AD and CB by the interchange of segments B and D (or A and C). These segments are not homologous. An interchange of non-homologous segments of two chromosomes is known as a *segmental interchange* or a mutual *translocation.* The first known translocation was discovered by Bridges in Drosophila. It arose spontaneously (apart from artificial treatment), but spontaneous translocations are comparatively rare.

The coming together of segments after chromosome breakage is a purely accidental matter. Hence in Fig. 83, left, segments B and C might have become interchanged (instead of B and D).

Moreover, the breakage itself might occur anywhere along the length of a chromosome, and so might result in segments of unequal length.

A translocation might involve the invisibly small tip end of one chromosome and a large segment of another, as segments B and D in Fig. 83, right. In this event it might appear as though the larger segment (B) had become attached to the intact end of the second chromosome (C). But actually, a broken off segment of one chromosome seldom if ever becomes attached to an intact end of another. The second as well as the first chromosome must be broken before a translocation can occur and then only the broken and sticky ends of the segments can unite. An intact chromosome apparently is enclosed in an envelope somewhat like a sausage casing and this prevents the attachment of a fragment either to the intact end or side.

Translocation Heterozygotes.—Suppose now that a sperm cell with a translocation fertilized a normal egg in Drosophila (Fig. 84, top). The fertilized egg would contain both the normal and the translocated chromosomes, and the fly which develops from it would be known as a *translocation heterozygote.*

When the reduction division takes place in the above translocation heterozygote, the chromosomes do not come together in pairs as normally, but instead they form a four-armed figure (Fig. 84, middle). The arms radiate from a common point, and each arm consists of a pair of homologous segments. This is the only way in which homologous segments of all four chromosomes can pair throughout their length. The chromosomes next separate at their mid-regions (because of terminalization of chiasmata), but they remain attached for a time at their ends. As a result they form a circle. Within the circle the normal chromosomes $(AB$ and $CD)$ alternate with the translocated $(AD$ and $CB)$. Next, the circle might get twisted in the form of an 8, and when this happens *alternate* chromosomes of the former ring face the same pole at metaphase. As a result the two normal chromosomes $(AB$ and $CD)$ go to one gamete and the two translocated $(AD$ and $CB)$ to the other (Fig. 84, bottom left). However, the circle might not twist into an 8. In this event adjacent chromosomes face the same pole at metaphase. It might happen that AB and CB face one pole and AD and CD the other (Fig. 84, middle bottom). Accordingly, AB and CB would go to one gamete, AD and CD to

the other. On the other hand, *AD* and *AB* might face one pole,
CD and *CB* the other, and the two corresponding classes of gametes
would then be formed (Fig. 84, bottom right).
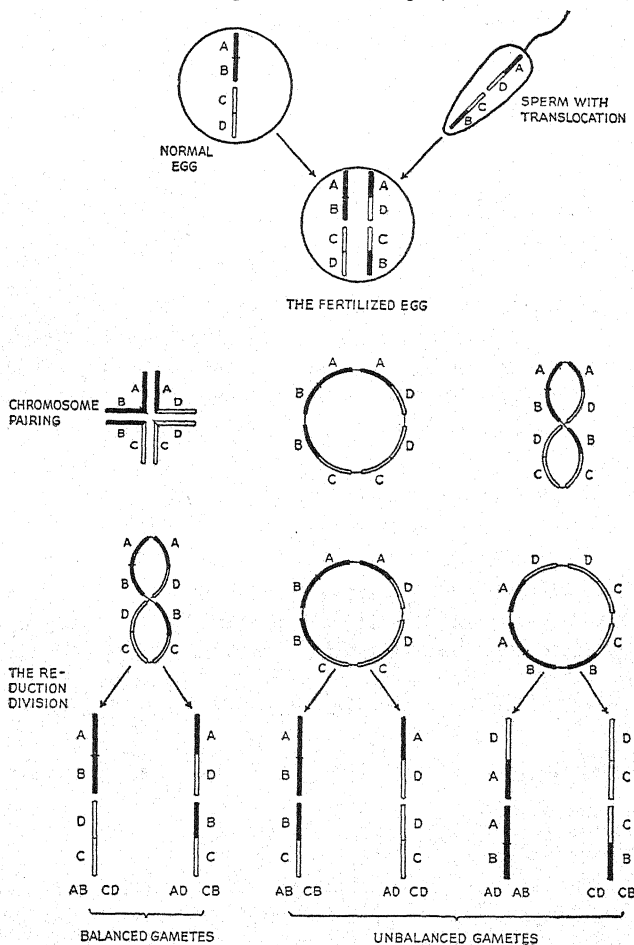
FIG. 84. A translocation heterozygote.

Whenever two adjacent chromosomes in a ring go to the same
pole, the resulting gametes contain one normal and one translo-
cated chromosome, as *AB* (normal) and *CB* (translocated). A
gamete with *AB* and *CB* lacks segment *D* but has a double dose
of segment *B*. Such a gamete is said to be a *deficiency* for *D* and

a *duplication* for *B*. The translocation heterozygote forms six classes of gametes, but only two of these contain neither a deficiency nor a duplication; namely, the class with the two normal chromosomes (*AB CD*) and the class with the two translocated (*AD CB*). We shall refer to these two classes as *balanced gametes*, and to the remaining four (the deficiency-duplications) as *unbalanced*. It should be noted that a balanced gamete contains one and only one segment of each kind.

If an unbalanced sperm cell, say, of composition *AB BC* should fertilize a normal egg (*AB CD*), an offspring would be formed of composition $\dfrac{AB\ BC}{AB\ CD}$. This would have only one dose of segment *D* but three of segment *B* and would be *genically unbalanced*. It would be incapable of development (inviable) because of the genic unbalance. In general, any gamete which contains a deficiency and a duplication (if large) would be incapable of giving rise to viable offspring on combining with a normal gamete at fertilization, for it would in every instance result in genic unbalance.

The Genetic Detection of Translocations.—It is possible to detect translocations by genetic methods. We might take as our example the detection of a translocation between the second and third chromosomes of Drosophila. The second chromosome of Drosophila contains the recessive mutant gene vestigial wings (*vg*); the third contains the recessive mutant spineless (*ss*, absence of the larger bristles). A vestigial spineless fly is $\dfrac{vg\ ss}{vg\ ss}$; a pure normal (long-winged, not-spineless) is $\dfrac{+\ +}{+\ +}$. A cross of the two $\left(\dfrac{vg\ ss}{vg\ ss} \times \dfrac{+\ +}{+\ +}\right)$ would produce F_1 offspring of genotype $\dfrac{vg\ ss}{+\ +}$, and the F_1 would normally form four classes of gametes: the two old combinations (*vg ss* and + +) and the two recombinations (*vg* + and + *ss*). Accordingly if an F_1 ♂ were test crossed $\left(\dfrac{vg\ ss}{+\ +} \times \dfrac{vg\ ss}{vg\ ss}\right)$, he would ordinarily produce the corresponding four classes of offspring: the old combinations $\left(\dfrac{vg\ ss}{vg\ ss} \text{ and } \dfrac{+\ +}{vg\ ss}\right)$ and the two recombinations $\left(\dfrac{vg\ +}{vg\ ss} \text{ and } \dfrac{+\ ss}{vg\ ss}\right)$. But suppose now

that in the F_1 male the two chromosomes with *vg* and *ss* were un-translocated (*AB CD*) and the two with the + alleles were translocated (*AD CB*), as shown in the upper part of Fig. 85. Then the F_1 male would form only two balanced classes of sperm cells; namely, *vg ss* and + + (the classes with the two normal chromosomes *AB CD* and the two translocated *AD CB*). The F_1 male would also form the two recombination classes *vg* + and *ss* + (or *AB*
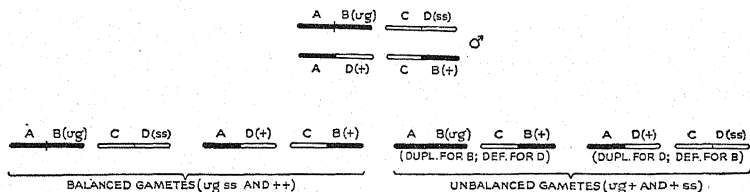
Fig. 85. The genetic detection of a translocation. Above, a translocation heterozygote of genotype $\dfrac{vg}{+}\ \dfrac{ss}{+}$; below, its gametes.

CB and *AD CD*), but these would be unbalanced. Hence if the F_1 male were crossed to a vestigial spineless female $\left(\dfrac{vg}{+}\ \dfrac{ss}{+}\ \sigma \times \dfrac{vg}{vg}\ \dfrac{ss}{ss}\ \varphi\right)$, only two classes of offspring would be produced capable of survival; namely, $\dfrac{vg}{vg}\ \dfrac{ss}{ss}$ (vestigial spineless) and $\dfrac{+}{vg}\ \dfrac{+}{ss}$ (long-winged and not-spineless). The recombination classes ordinarily expected would be $\dfrac{vg}{vg}\ \dfrac{+}{ss}$ and $\dfrac{+}{vg}\ \dfrac{ss}{ss}$, or vestigial not-spineless and long spineless. But these would fail to develop because they are genically unbalanced. Thus the absence of these recombination classes from among the offspring of the test cross would indicate that the F_1 male was heterozygous for a translocation between the second and third chromosome.

Suppose now that we X-rayed some normal flies and wanted to find out whether we produced any translocations between the second and third chromosomes. Then we might mate the X-rayed flies to vestigial spineless $\left(\dfrac{+}{+}\ \dfrac{+}{+} \times \dfrac{vg}{vg}\ \dfrac{ss}{ss}\right)$, in order to get F_1 males of genotype $\dfrac{vg}{+}\ \dfrac{ss}{+}$. Any of these in which the treated chromosomes (+ +) were translocated would upon being test-crossed form

only two classes of offspring instead of four, and we should thereby know that they carried a translocation between the second and third chromosomes.

In the above cross the classes that fail to appear are the *recombination* classes (vestigial not-spineless and long spineless). In effect, therefore, vestigial and spineless are completely linked in the translocation heterozygote, even though they are in separate chromosomes.

Genetic Identification of Unbalanced Gametes in a Translocation Heterozygote.—The above test cross might be interpreted to mean either that the translocation heterozygote forms only the two balanced classes of gametes (*AB CD* and *AD CB*), or that other classes are also formed but are incapable of producing viable offspring upon combining with normal gametes. In some forms of life (such as the evening primrose), only the two balanced classes are formed by a translocation heterozygote. But in Drosophila the unbalanced classes are also formed (containing duplications and deficiencies). The gametes in question actually survive and are capable of combining with other gametes in fertilization. But when they combine with normal gametes (as in the above test cross), they produce genically unbalanced offspring, and these are incapable of development. This can be shown as follows.

According to Fig. 84 (lower middle part) the translocation heterozygote represented in the figure produces one class of gametes of composition *AB CB*, another of composition *AD CD*. The first (*AB CB*) has two doses of *B* but none of *D;* the second (*AD CD*) two doses of *D* but none of *B*. These two classes of gametes are known as complementary duplication-deficiencies, because each one has in extra amount what the other lacks. (There are two other such classes, shown at bottom right in Fig. 84.) If two translocation heterozygotes interbreed, *AB CB* of one heterozygote might combine with *AD CD* of the other, giving a fertilized egg of composition $\frac{AB\ CB}{AD\ CD}$. This egg contains two normal chromosomes (*AB CD*, diagonally opposite in the above formula) and two translocated (*AD CB*, also diagonally opposite). It is genically balanced, and if it is produced it should develop.

Assume now that two translocation heterozygotes interbreed, both of composition $\frac{AB\ CD}{AD\ CB}$, but that one contains *vg* in each *B*

segment and the other ss in each D segment, thus: $\dfrac{AB(vg)}{AD}\dfrac{CD}{CB(vg)}\times$

$\dfrac{AB}{AD(ss)}\dfrac{CD(ss)}{CB}$. Assume further that the first parent produces some gametes of class $AB(vg)\ CB(vg)$ and the second parent some of class $AD(ss)\ CD(ss)$. If these gametes combined they would produce viable offspring of genotype $\dfrac{AB(vg)}{AD(ss)}\dfrac{CB(vg)}{CD(ss)}$. These would appear vestigial spineless. No other combination of gametes could produce vestigial spineless. According to Dobzhansky, vestigial spineless offspring would actually be produced by the above mating, and this shows that a translocation heterozygote in Drosophila produces functional gametes with a deficiency and a duplication.

Translocation Heterozygotes in Plants and Animals Compared.—In plants pollen cells must grow down the pistil before they can produce the sperm cells. Pollen cells which contain duplications and deficiencies are usually incapable of growth and therefore are sterile. Translocations occur in corn and when a plant is heterozygous for a translocation, about 50 per cent of its pollen grains are shriveled up and sterile. These are the grains with deficiencies and duplications. The same sort of thing applies to the megaspores (the cells which give rise to the eggs).

The mature sperm and egg cells of animals do not undergo any extensive growth before fertilization; hence they can survive and function in fertilization even though they are genically unbalanced. It is to be remembered in this connection that in many species of animals half the sperm cells normally lack an X and are genically unbalanced, yet they survive and are as capable of fertilizing eggs as are the X-containing sperm cells. The reason for this is that the mature sperm cells undergo no growth whatever (before fertilization), and it is only for growth and development that genic balance is necessary.

Locating the Breakage Points in a Translocation.—A translocation includes breaks in two chromosomes, and we can refer to the points where the breaks occur as the *breakage* or *translocation points*. Thus if the normal chromosomes are $ABCD$ and $EFGH$ and the translocated $ABGH$ and $EFCD$, then with reference to the normal chromosomes the breakage points are between B and C and between F and G.

It is possible by means of linkage experiments to locate the breakage points in a translocation. Thus suppose that a transloca-tion heterozygote were of genotype $\dfrac{a\,b\,c\ d\ e\,f\,g\,h}{ABGH\ EFCD}$ (Fig. 86, left). Then the breakage points are between loci b and c in one chromo-some and between f and g in the other (indicated by lines at the base of the arms in Fig. 86). Inspection of Fig. 86, middle, will make it evident that a crossover between locus b and the breakage point would result in crossover chromosomes of composition $abGH$ and $ABcd$. Therefore if the heterozygote produced gametes
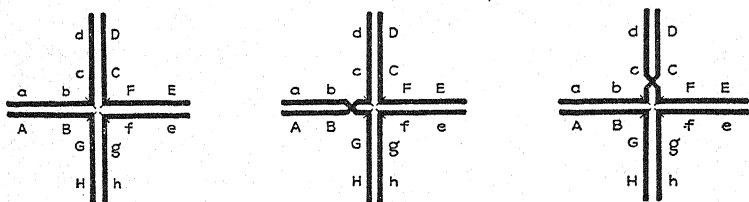
FIG. 86. The genetic detection of the breakage points in a translocation.

with either of these combinations of genes we should know that the translocation point was to the right of locus b. Such combi-nations could be identified by means of a test cross. In like man-ner, a crossover between locus c and the translocation point (Fig. 86, right) would give chromosomes of composition $dcFE$ and $DCba$ (again identifiable by means of a test cross), and these combinations would tell us that the translocation point was to the left of locus c. Since the breakage point is to the right of b and to the left of c, it must be between loci b and c. In a similar manner we could determine where the translocation point was in chromo-some $efgh$.

Translocations in Nature.—Suppose that $1\cdot2$ and $3\cdot4$ were two normal chromosomes and $1\cdot4$ $3\cdot2$ two translocated, say, in some plant. The heterozygote would be $\dfrac{1\cdot2\ \ 3\cdot4}{1\cdot4\ \ 3\cdot2}$. It would produce some gametes of composition $1\cdot4$ $3\cdot2$, and these by combining might give rise to a race homozygous for the new segmental arrangement $\left(\dfrac{1\cdot4\ \ 3\cdot2}{1\cdot4\ \ 3\cdot2}\right)$. Translocations might theoretically take place between any two chromosomes in a given species, and so

numerous races might come into existence differing in the segmental arrangement of their chromosomes. This sort of thing has happened in many species, but it has been studied in particular by Blakeslee and Belling in the Jimson weed (Datura). There are twelve chromosomes in Datura (haploid number) and in the normal or *standard* race they have been numbered 1·2 3·4 5·6, etc. A translocation might involve any two of the twelve. The standard race, or any race derived from it by a single translocation, is known as a *prime type*. Combinations of derived prime types are possible, and these give rise to more complex segmental rearrangements, as for example a combination produced by the crossing of 1·4 3·2 5·6 7·8 and 1·2 3·4 5·8 7·6, giving 1·4 3·2 5·8 7·6 as a recombination possibility. The derived prime types do not as a rule differ from the standard race in appearance. Nevertheless they are to be regarded as mutants in the broadest sense of the term, insofar as they are different from the normal race in the arrangement of their chromosome segments, and this difference is hereditary.

Suppose now that a derived prime type (say, 1·4 3·2) were crossed to the standard (1·2 3·4). Then the offspring would be

a. THE CHROMOSOMES
BEFORE RING FORMATION

b. THE PAIRING OF
LIKE ENDS AT THE
REDUCTION DIVISION
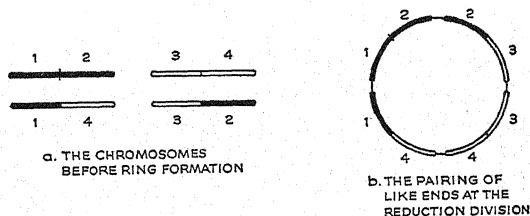
FIG. 87. Ring formation in a Datura translocation heterozygote.

$\dfrac{1·4\ \ 3·2}{1·2\ \ 3·4}$, and at the reduction division the chromosomes would form a ring as shown in Fig. 87. This ring formation is due to the fact that like segments attract each other, essentially as happens in the case of a Drosophila translocation heterozygote.

If two plants were crossed and each had the same segmental arrangement, say, $\dfrac{1·4\ 3·2}{1·4\ 3·2}$, then the offspring of course would also be $\dfrac{1·4\ \ 3·2}{1·4\ \ 3·2}$, and since the chromosomes run in perfectly matched

pairs, they would pair in the ordinary manner at the reduction division. In order to tell, then, whether two Datura plants belong to the same prime type or to different ones, we breed them together and examine the chromosomes of the offspring at the reduction division. If all the chromosomes come together in two's in the offspring, the parents belong to the same prime type; if some of the chromosomes form a ring, then the parents belong to different prime types. After having thus distinguished the various prime types from one another, we could give them numbers. The standard or normal race has been arbitrarily assigned the number 1, and the derived prime types have been numbered 2, 3, 4, etc. Over forty prime types have been found in a state of nature.

It is possible to tell which chromosomes have become translocated in Datura, for in the hybrid formed by crossing the standard and a derived prime type, a ring of chromosomes is always formed, and the ring contains not only the translocated chromosomes but also the standard chromosomes from which the translocated were derived. The standard chromosomes of Datura differ in size and shape and so can be distinguished apart under the microscope. Hence it is often possible to identify the standard chromosomes in a ring, and so to determine which chromosomes have undergone translocation.

Datura is a weed of extremely widespread distribution, being found in almost all parts of the world largely because of its accidental distribution with exported farm products which it contaminates. Most of the Daturas the world over fall into five prime types. A given prime type usually occupies a fairly extensive geographical area to the exclusion of other prime types. In Europe and Asia the prevalent prime type is one that has been given the number 2. In South America there are principally four prime types (1, 2, 3, and a combination of 2 and 3), and each of these occupies certain areas. The different prime types are probably not specifically adapted to different geographical regions. The spread of a given prime type in a given region was probably accidental. The study of prime types in Datura shows that translocations occur in nature, and that they might give rise to races which comprise large sections of a natural population.

There are over five hundred varieties of Datura in nature, differing in flower color, shape of leaves, and in other visible

traits. The differences between the varieties of Datura are for the most part due to gene mutations and have as a rule nothing to do with differences in arrangements of chromosome segments.

Inversions.—Suppose that we picked out four genes along the length of a chromosome and lettered them *ABCD*. Then by means of X-rays it would be possible to break the chromosome into three segments *A*, *BC*, and *D*. The middle segment might then become reversed and attached to the end segments, giving rise to the rearrangement *ACBD*. This is known as an *inversion.*

When a chromosome is broken into just two segments, it never happens that one of the segments then becomes inverted. Inversions always involve two breaks, and it is always the internal segment which becomes inverted, never one of the external segments. Only the broken and sticky ends of chromosome segments can become attached after breakage, as previously mentioned. The unbroken ends contain a covering or cap (the *telomere*) which prevents them from sticking to one another or to a broken end.

Inversions vary greatly in size. Some involve just a small segment of a chromosome, others a very large segment, and there are all intermediate sizes. The very large ones might stretch almost from one end of a chromosome to the other end, leaving only a small external segment at each end.

Inversion Heterozygotes.—If a sperm cell with an inversion (*ACBD*) fertilizes a normal egg (*ABCD*) an offspring is produced
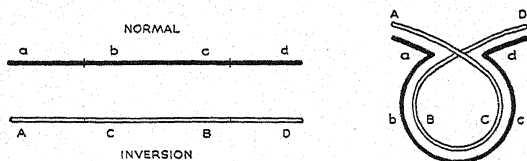
Fig. 88. An inversion heterozygote. Left, the chromosomes before synapsis; right, synapsis.

which contains both the inverted and the normal chromosomes, thus $\dfrac{ACBD}{ABCD}$. This offspring is heterozygous for the inversion and can be referred to as an *inversion heterozygote.* If the above normal egg (with the uninverted chromosome) had contained the recessives *abcd* instead of the dominants *ABCD*, the offspring would have been of composition $\dfrac{ACBD}{abcd}$; that is to say, it would have been

heterozygous both for the inversion and for the genes at loci *abcd.*

At the reduction division the inverted and the normal chromosomes pair in an inversion heterozygote. But the only way in which they can pair exactly (gene for gene) is by forming a loop in the manner shown in Fig. 88.

The Suppression of Crossovers Within the Limits of an Inversion.—In Fig. 89 a crossover is shown within the limits of the inversion in a heterozygote of composition $\frac{a\,b\,c\,d}{A C B D}$. The circle at one end of each chromosome (near *a* or *A*) represents the point of attachment of the spindle fiber (the centromere). It will be seen that where the crossing over occurs segment *ab* connects with segment *CA*, giving chromosome *abCA;* and *DB* connects with *cd*, giving *DBcd*. The two crossover products are shown in the lower part of the figure. The first of the two (*abCA*) has two centromeres and is known as a *dicentric* chromosome ("centric" standing for "centromeric"). The second crossover product (*DBcd*) has no centromere and is known as an *acentric* chromosome.
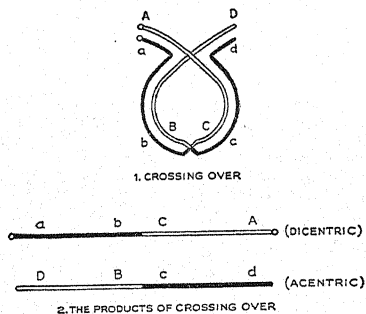
When cell division takes place the two ends of chromosome *ACba* are pulled in opposite directions, for each end has a centromere and a spindle fiber, and the spindle fibers draw the ends toward opposite poles. The result is that chromosome *abCA* remains in the middle of the dividing cell. Moreover, chromosome *DBcd* lacks a centromere and therefore also remains in the middle of the dividing cell. Hence when the reduction division completes itself, the crossover products are partly in both the daughter cells formed by the division, and so they get lost.

1. CROSSING OVER

2. THE PRODUCTS OF CROSSING OVER

FIG. 89. Crossing over within the limits of an inversion in an inversion heterozygote.

The above account is somewhat simplified. Before crossing over takes place between *abcd* and *ACBD*, each chromosome splits, giving four strands or a tetrad (Fig. 90-1). Sister strands are held together as usual by the centromeres (shown as circles in Fig. 90-1).
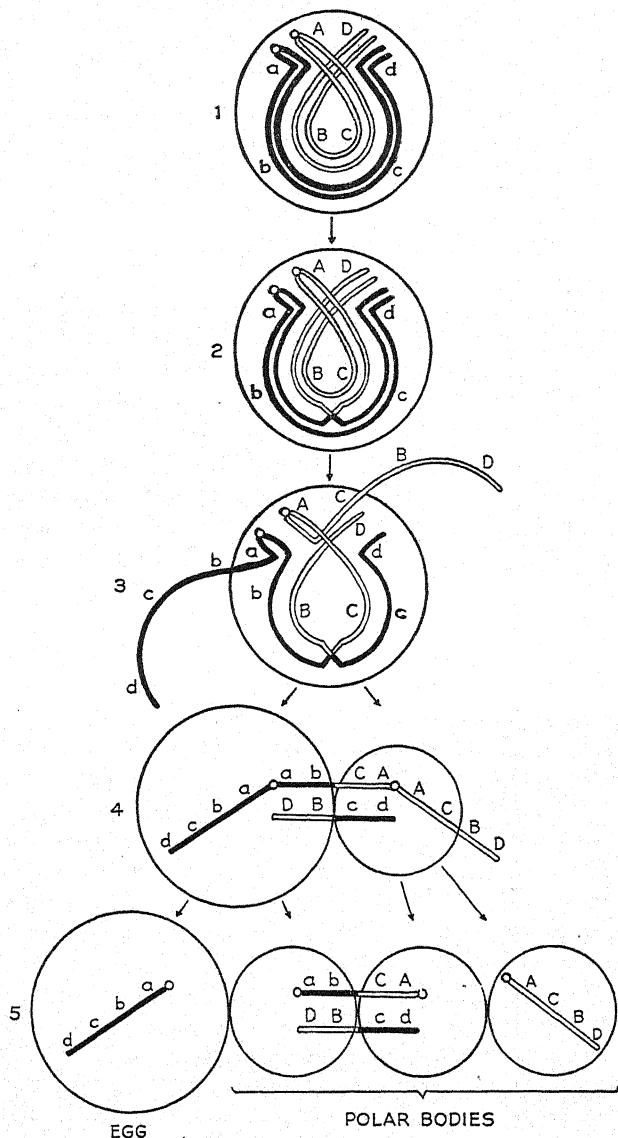
Fig. 90. The apparent suppression of crossing over by an inversion. Stage 1, the tetrad before crossing over; 2, crossing over between two of the strands (non-sister); 3, the two non-crossover strands separated from their sister strands (for the sake of clarity); 4, first meiotic division completed, with non-crossover strands at the end of a chromatid tie; 5, the second meiotic division completed. The egg contains a non-crossover strand (as does also the end polar body).

Crossing over now takes place between one of the strands of *abcd* and one of *ADCB* (Fig. 90-2). The other strand of *abcd* and of *ACBD* is not necessarily involved in crossing over and often would not be. One of the crossover chromosomes is *abCA* (Fig. 90-3). This has both centromeres and hence both the non-crossover sister strands are connected with it. The non-crossover strand *abcd* is connected with the *a* end of *abCA* and the non-crossover strand *ACBD* is connected with the *A* end of *abCA*. Thus at the completion of the reduction division the two non-crossover strands are connected by means of the dicentric crossover chromosome (Fig. 90–4). This connection is known as a *chromatid tie*. At the equation division, the centromere at each end of the chromatid tie divides, and the *non-crossover* strands are drawn into the outer two of the four cells formed (Fig. 90-5). As the egg is one of these outer cells, it will always receive one or the other of the non-crossover strands. Thus it happens that in an inversion heterozygote the crossovers get lost in the polar bodies, and the eggs always receive the non-crossovers when single crossing over takes place within the limits of the inversion.

A double crossover within the limits of the inversion does not form a chromatid tie in an inversion heterozygote. This can be seen by inspection of Fig. 91. Here one of the products of double crossing over is *abCDef*, and the other *AEdcBF*. Each of these chromosomes has just one centromere, not two. Hence they are drawn to opposite poles just as though they were non-crossovers. But the double crossovers within an inversion are comparatively few, especially if the distance covered by the inversion is small. Hence crossing over is in effect suppressed within the limits of an inversion.

One could determine the length of an inversion by noting the length of the chromosome segment over which single crossovers were suppressed. To do this it would be necessary to get a female heterozygous both for the inversion and for mutant genes conveniently located along the length of the chromosome in the expectation that some of them would be inside the inversion and some outside, as in the inversion heterozygote $\dfrac{a\,b\,c\,d}{A\,C\,B\,D}$. We should not know in advance that the section of the chromosome carrying genes *CB* had been inverted and that *A* and *D* were outside of the inversion, but by selecting the mutant genes *abcd* over the entire length of the chromosome we should in the present example get

two (b and c) which happened to be within the limits of the inversion and two others (a and d) which happened to be outside of these limits.

The apparent suppression of crossing over in an inversion heterozygote was discovered before the inversion itself was recognized as such. That is to say, flies were got hybrid at several loci in the same chromosome pair, and it happened that one of the members

1. DOUBLE CROSSING OVER

2. THE PRODUCTS OF DOUBLE CROSSING OVER
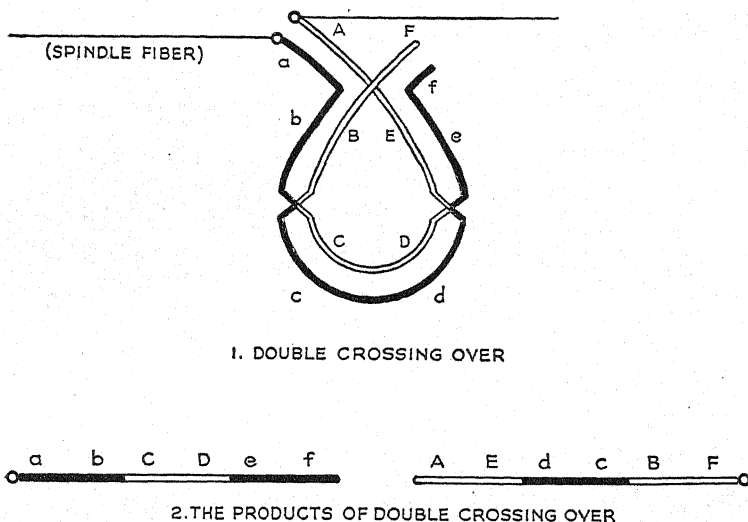
FIG. 91. Double crossing over within the limits of an inversion in an inversion heterozygote.

of the pair contained an inversion which arose accidentally, but which the experimenter was not aware of. On making a test cross of the hybrid in question, an absence of crossover classes was noticed among the offspring. The cause of the suppression in crossing over was at first unknown, but it was referred to as a "crossover suppressor" or "little crossover factor," and was given the symbol C (the initial of crossover). These terms are still in use, although it is now known as a result of linkage studies by Sturtevant that the absence of normally occurring crossover classes is usually due to an inversion.

Overlapping Inversions.—Sometimes a second inversion takes place in a chromosome which already has one, giving a compound inversion. Thus in Fig. 92-1 we begin with a normal chromosome

with genes *a–m* (in alphabetical order). Suppose now that segment
c to *h* were inverted (the short cross lines between *b* and *c* and
between *h* and *i* indicating the breaks that result in the inver-
sion). This first inversion would give the rearrangement shown
in Fig. 92-2. In the inversion heterozygote the inverted segment
would be included in the loop shown in Fig. 92-2.1. The heterozy-
gote would of course contain both the normal and the inverted
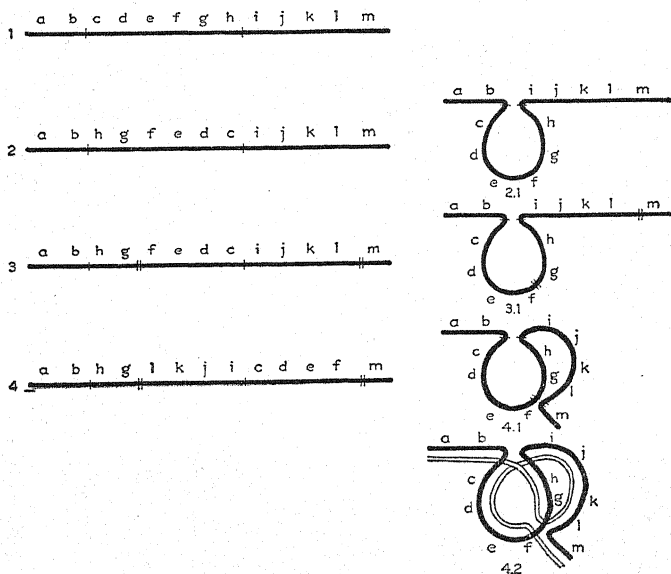
FIG. 92. Overlapping inversions.

chromosome, but in Fig. 92-2.1 we are indicating only the normal
chromosome. The inverted would go from *a* to *b* and then cut
across to *h g*, etc., since the inverted chromosome is *a b h g*, etc.
In the formation of the loop shown in Fig. 92-2.1 the two breaks
(indicated by the short cross lines) are brought close together.
Assume next that a second inversion takes place and that the left
break for this inversion comes between *g* and *f* of Fig. 92-2, and
the right break between *l* and *m*, as indicated by the short double
lines in Fig. 92-3 and 92-3.1. This second inversion is said to
overlap the first. It would give us the chromosome shown in Fig.
92-4 and is got by inverting the entire section shown between
the short double lines. In the inversion heterozygote the short

double lines shown in Fig. 92-3.1 would be brought together, giving us the compound loop shown in Fig. 92-4.1. We next add the inverted chromosome to the double loop shown in Fig. 92-4.1. This is done by first taking segment *ab* of Fig. 92-4 and indicating it as a light segment beside *ab* of Fig. 92-4.2 (heavy). Next *hg* of Fig. 92-4 (light) is placed beside *hg* of Fig. 92-4.2 (heavy) so that chromosome *a b h g*, etc., cuts across from *b* to *h*. In Fig. 92-4
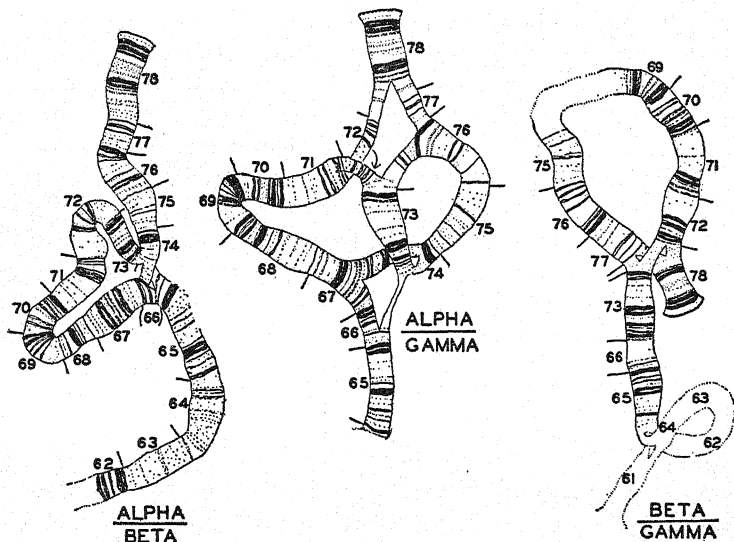
FIG. 93. Simple and compound inversions in *Drosophila azteca*, as shown by salivary gland chromosomes. See Fig. 92 for the origin of the compound inversion. (From Dobzhansky and Socolov in *The Journal of Heredity*.)

segment *hg* is followed by *l k j i*, and this is next added to Fig. 92-4.2. By continuing, we finally arrive at the compound loop shown in Fig. 92-4.2. This would be produced by an inversion heterozygote which contained the normal chromosome (Fig. 92-1) and the one with the double inversion (Fig. 92-4).

If we crossed two races, say, *A* and *B*, and if the heterozygote formed a simple loop of the kind shown in Fig. 92-2.1, we should know that the two races differed by a simple inversion. On the other hand, if we crossed two races, *A* and *C*, and if the heterozygote formed a compound loop of the kind shown in Fig. 92-4.2, we should know that the two races differed by two overlapping

inversions. If now we regard one of the races, say, A, as normal, then we know that C was probably derived from A by two inversions, and that the evolutionary order of the three races concerned was $A B C$; that is, C was derived from A through B. Of course race B crossed to C would give a simple loop, since B and C differ by only one inversion.

In Drosophila the chromosomes in the salivary glands are very large, and they contain cross bands which are characteristic for different parts of the chromosome (Fig. 93). Moreover, the chromosomes pair just as they do in the reproductive organs before gamete formation. Hence it is relatively easy to detect loop formation in the salivary glands of an inversion heterozygote and to determine precisely the location of the breaks which produced the inversion. Figure 93, left, shows a simple loop essentially as shown in Fig. 92-2.1 and was produced by crossing two races (A and B) which differ by a single inversion. Figure 93, middle, is a compound loop, similar to Fig. 92-4.2 and was produced by crossing race A with another race (C) presumably derived from A through B by a second inversion. In Fig. 93, middle, we get the normal segmental order by reading the numbers in order, beginning with 65.

Inversions in Nature.—Inversions occur in a state of nature and lead to racial differences. This has been clearly shown by studies made by Dobzhansky on *Drosophila pseudo-obscura*, a species of Drosophila which inhabits western North America. Specimens of *D. pseudo-obscura* collected from different regions in California and Mexico often differ in the arrangement of the genes in a given chromosome in such a way that if one arrangement of genes is regarded as normal or standard, it is possible to derive the other arrangements from this by one or more inversions (Fig. 94). These inversion races are limited to definite localities, and they have been named for the most part after the locality which they inhabit, as Pikes Peak, Estes Park, Cuernavaca. It is possible to arrive at the evolutionary relationships of the various inversion races by means of *overlapping inversions*, as explained above. For example, if we proceed upwards from standard in Fig. 94, we come first to "hypothetical A," which has not yet been found (perhaps it has died out) and then to "Santa Cruz," which when crossed to standard gives a compound loop of the kind shown in Fig. 92-4.2 and which therefore differs from standard by two inversions, the first of which according to Dobzhansky was the one

that gave rise to "hypothetical *A*." Santa Cruz in turn gave rise, by single inversions, to four separate races. One of these four (Tree Line) next gave rise to four other races (Olympic, Estes Park, Oaxaca, Hidalgo) by means of a second inversion (after the Santa Cruz stage), the second in each case "overlapping"
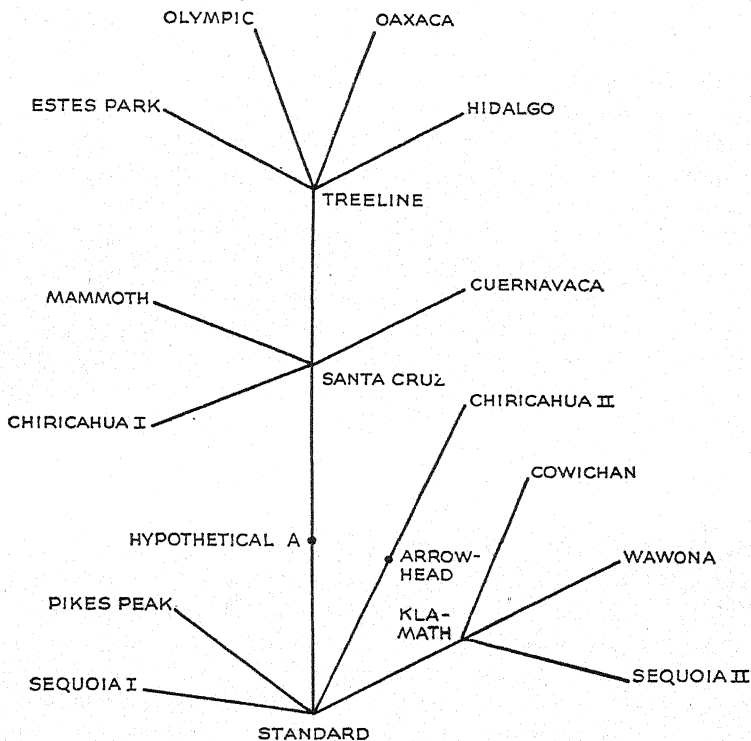
FIG. 94. The evolutionary relationships of races produced by inversions (in the third chromosome) of *Drosophila pseudo-obscura*. (After Dobzhansky and Sturtevant in *Genetics*.)

the first inversion. This is shown by the fact that each of the four last races, when crossed to Santa Cruz, gives a compound loop, one of the components of which is the inversion that produced Tree Line (the race from which the other four were presumably derived). In like manner the rest of Fig. 94 is derivable from the standard race.

Deletions.—When a chromosome is broken into three segments, it is a mere matter of chance whether or not the internal segment

will again join up with the two external segments. Sometimes the two end segments become directly connected, and the middle segment is omitted or *deleted*, as shown in Fig. 95. A chromosome from which an internal segment has been removed is said to have a *deletion*. X-rays readily break up chromosomes into segments; hence they readily produce deletions (along with other chromosomal rearrangements). But deletions also turn up occasionally in the absence of any treatment in both plants and animals.

Deletion heterozygotes are genically unbalanced because they have only one dose of every gene in the region of the deletion as compared to two elsewhere. In case the deletion were large, the
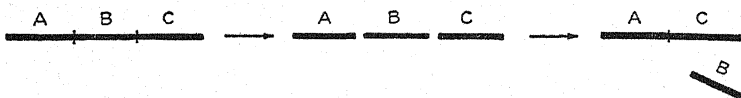
Fig. 95. A deletion.

heterozygote could not develop because of genic unbalance. Offspring that were *homozygous* for a large deletion could not develop because they would completely lack the genes in the region of the deletion, and any large deletion would as a rule contain one or more genes necessary for life.

Small deletions might not prevent development but often they cause the adult to have some mutant trait. Thus in Drosophila a small deletion in a certain part of the *X* chromosome causes the tips of the wings to be notched and is referred to as "Notch." This deletion has been observed to arise rather often (over 50 times) and varies somewhat in length in deleted *X*'s of independent origin, but usually it is about 1.5 map units in length, and it always includes the locus of "facet" (rough eyes, located at point 3 on the chromosome map) and sometimes includes also the locus of white (located at point 1.5). Notch flies are never males because the males carry only one *X* and if this should contain the Notch deletion, the fly would completely lack the deleted segment and would fail to develop. In the female however there are two *X*'s, and if one should have the deletion, the other might be normal and the fly would develop. Females heterozygous for the deletion are Notch and the deletion therefore is dominant as regards its influence on wing development.

In Drosophila again, small deletions often cause a reduction in bristle size (minute bristles) and are known as "Minutes."

Over seventy-five different minutes have been found, distributed in all four chromosomes of Drosophila. Homozygous minutes cannot live. The heterozygotes appear minute, so that the deletion is dominant for its visible effect. It is rather odd that small deletions should influence the bristles so often. Undoubtedly they also influence vital organs but are then lethal.

If a deletion is very small it might not prevent development even when homozygous, and it might then produce some mutant trait. In Drosophila yellow body has arisen several times by mutation and ordinarily is not regarded as a deletion effect, but in one instance it has been found to be due to a small deletion large enough to be microscopically observable. It is conceivable that the remaining yellows are due to deletions that are too small to be visible. Just how often mutations represent minute deletions is not known.

The Formation of Attached X's.—In Drosophila the X chromosome has a centromere at one end (the "right" end), and at this end it also has a large inert segment which is homologous with the inert material in the Y. As a result of rare crossing over
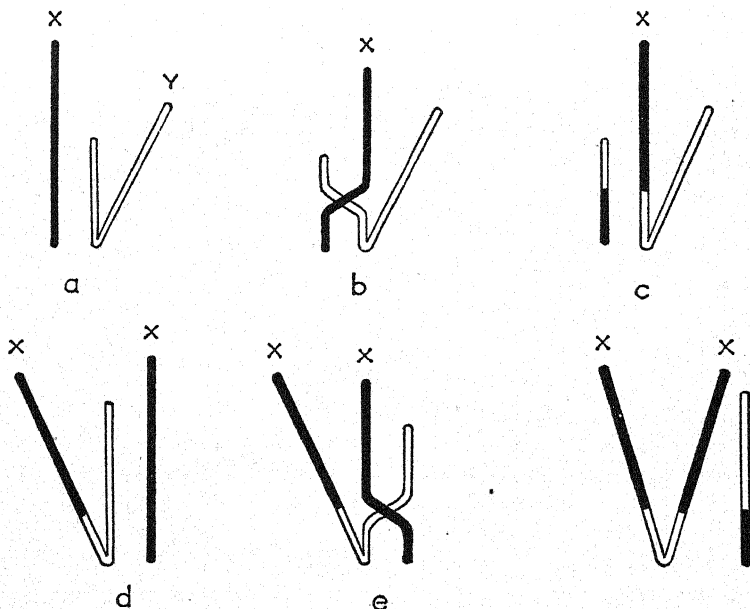
Fig. 96. The production of attached X's.

an arm of the Y might come to be attached to the X (Fig. 96a, b, c). Crossing over between a normal X and the Y-segment of the X-Y compound might then result in "attached X's" (Fig. 96d, e, f).
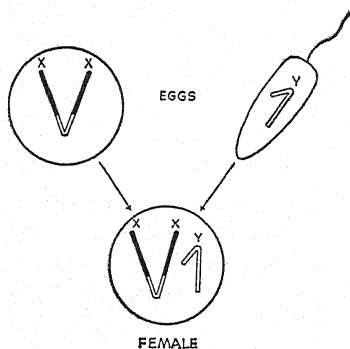
FIG. 97. The fertilization of an attached-X egg by a Y sperm.

If an egg with attached X's were fertilized by a Y-containing sperm cell, the resultant offspring would have two X's (attached) and a Y (Fig. 97). This would be an attached-X female. It would be normal-appearing, the Y having little or no influence on her development, since it is largely inert.

The X and the Y are not strictly homologous (throughout their entire length) and crossing over between them might therefore be regarded as the equivalent of a translocation. Attached X's are, then, the result of two translocations between the X and the Y.

Duplications and Repeats.—If we treated a normal male Drosophila with X-rays we might by chance strike an X chromosome in a sperm cell and produce a large deletion (Fig. 98, upper half). The deleted X would therefore contain only a small segment of active chromatin (segment A at the left end of the X). If the treated male were mated to an attached-X female, the sperm cell with the deleted X might happen to fertilize an egg with attached X's, giving an offspring with attached X's and
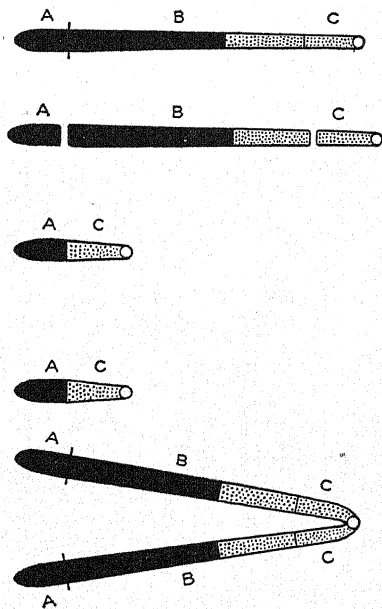
FIG. 98. A duplication. Dark is active chromatin; stippled, inert.

the deleted X in addition (Fig. 98, lower half). The deleted X would therefore be a duplication in that it contains a segment of the X (segment A) already present in double amount (in the attached X's). The duplicated segment would be distributed in the normal manner at cell division, since it has a centromere (derived from the right end of the X). If the duplication were comparatively small, it might not interfere with development but it might produce some mutant effect, such as small bristles, somewhat shortened wings, or rough eyes. Large duplications cause considerable genic unbalance and thus prevent development to the adult stage.

Suppose now that the attached X's in Fig. 98 were pure for a number of recessive genes, say, $y\,w\,cv$ (yellow, white, crossveinless), and that $y\,w$ were in segment A, cv in segment B. Suppose further that segment A of the duplication contained the normal alleles of y and w but that it did not contain the normal allele of cv because it did not extend to the locus of cv (the genotype of the fly being $\overbrace{\underset{\underset{y\quad w\quad cv}{\diagdown}}{\overset{+\quad +}{\underset{y\quad w\qquad cv}{\diagup}}}}$). Then y and w would not express themselves but cv would (one dose of a dominant usually being sufficient to suppress two doses of a recessive). It is evident from this that we can determine the size of a duplication by the length of the segment in which recessives can be suppressed by the duplication.

If we begin with two normal chromosomes, say, abc and de, segment b might be deleted from abc and inserted into de, giving $ac\,dbe$. If b were comparatively small, it might be possible to get offspring (by crossing and recombination) which were either $\dfrac{abc\;dbe}{abc\;d\;e}$ (with three doses of segment b) or $\dfrac{abc\;dbe}{abc\;dbe}$ (with four doses of b). Segment b in chromosome dbe would therefore be a duplication and it might have some mutant effect.

A small segment of a chromosome might sometimes be duplicated as the result of unequal crossing over, as shown in Fig. 99-1, 2, 3. The duplication in this case is known as a *repeat*. Further unequal crossing over might then take place within the repeat stock (Fig. 99-4, 5). This might result in two chromosomes: one having a second repeat and the other a reversion to normal. In

Drosophila the mutant Bar (narrow eyes) is due to a small repeat. This cannot be seen in the chromosomes as they are found in most parts of the fly, but in the salivary glands it happens that the
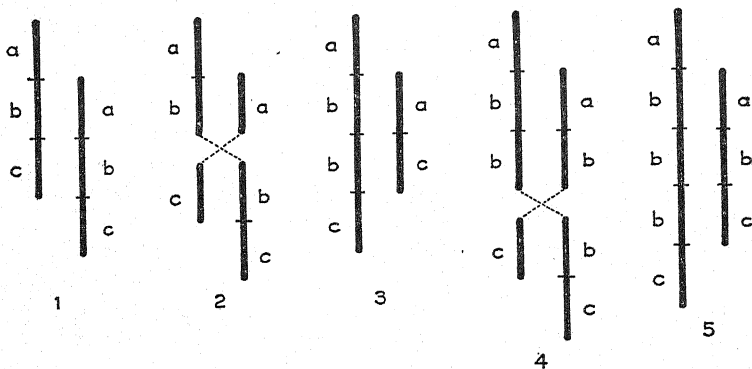
FIG. 99. Unequal crossing over.

chromosomes are unusually large and it is possible to see in them that a certain segment of the normal chromosome is repeated in Bar (Fig. 100, left and middle). Further unequal crossing over within the Bar stock might cause the repeat to be deleted, giving a reversion to the normal condition (called Bar-reverted); or still another repeat might be added to the chromosome, giving two repeats and resulting in the mutant known as "Bar-double."
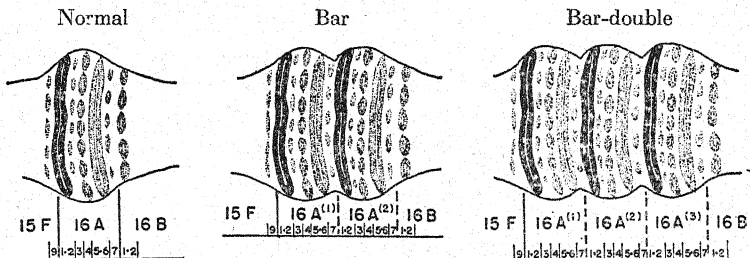
FIG. 100. A repeat in Drosophila (Bar eyes). (From Eileen Sutton in *Genetics*.)

Other mutants besides Bar are known to be due to small repeats and perhaps some mutants are due to repeats that are too small to be visible.

Ring Chromosomes.—When a chromosome is broken into three segments (an internal and two external), the internal seg-

ment has two broken ends, and if the two ends should happen to come together they would stick to each other and a ring-shaped chromosome would result (Fig. 101).
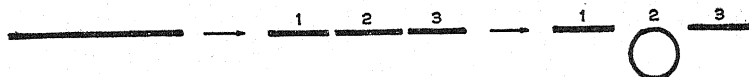
FIG. 101. The production of a ring chromosome.

In Drosophila a ring chromosome can be derived from attached X's in the manner shown in Fig. 102. In effect the ring is produced by a translocation between the tip of one X and the base of the other. The ring contains a duplication of the basal part of the X and a deletion of tip. But the duplication consists largely of inert material, and the deletion is very small; hence neither interferes with development. A stock of flies has been got with a ring X and is known as "closed X" (X^c).
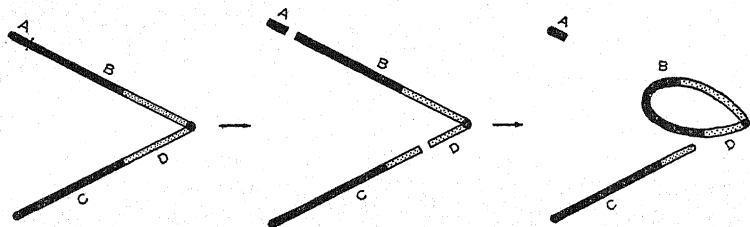
FIG. 102. The origin of a ring chromosome from attached X's in Drosophila.

At the reduction division ring chromosomes pair and split, forming a tetrad as usual. Fig. 103 (upper) shows a tetrad in which two of the ring-shaped strands have been lifted up for the sake of clarity, but they should be next to their sister strands (*abc* next to *abc*, and *ABC* next to *ABC*). Crossing over between two rings produces a dicentric ring of double size (Fig. 103, lower). The crossovers therefore result in a chromatid tie, and they get lost in the polar bodies, just as in the case of a chromatid tie in an inversion heterozygote. But the non-crossover sister strands are at the ends of the tie and one of them would always get into the egg, again as in the case of an inversion heterozygote. In plants crossing over between ring chromosomes would cause considerable pollen sterility (because of chromatid ties); it would also cause some male sterility in animals in which there was

crossing over in the male. Ring chromosomes are not found as normal constituents of a cell, possibly because of the sterility which they cause.
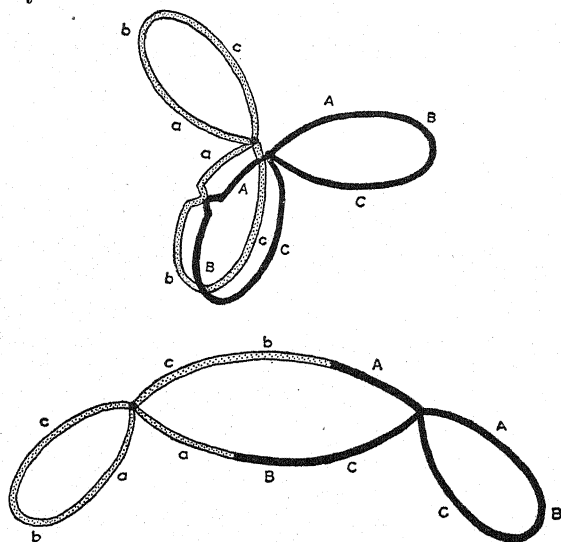
FIG. 103. Crossing over between ring chromosomes. (To derive the lower figure, begin at *a* in the upper figure, then cross over to *B*, *C*, etc.)

The Position Effect.—Translocations and inversions cause no loss or gain in amount of chromosome material but merely a segmental rearrangement of genes. Very often the rearrangement has no visible effect on development, so that adults with translocations and inversions appear perfectly normal. Sometimes, however, the rearrangement has a mutant effect. This is known as the *position effect*. Perhaps the chemical products of adjacent genes sometimes react immediately upon being formed but fail to interact when they are separated, or they interact with the chemical products of other genes, with a resultant position effect. In Drosophila the Bar eye mutation is a position effect and depends upon the changed position of the dark double band labeled $16A^{(2)}1 \cdot 2$ in Fig. 100 (middle). In the normal fly there is only one such double band, in section $16A^{(1)}$ and it is next to 15 $F9$. But in the duplication $16A^{(2)}$ the dark double band is next to $16A^{(1)}7$, and it is this altered position that causes the Bar eye effect, not the mere duplication of chromatin material. This can be shown

by removing section $16A^{(2)}$ to some other position (in an inversion or a translocation). Bar then as a rule reverts to normal, though the duplicated material is still present. But when duplications are large, they undoubtedly produce an effect in their own name, due to the genic unbalance which they cause.

The Mechanism of Chromosomal Rearrangements.—A translocation involves an interchange of chromosome segments and therefore is a form of crossing over, but unlike true crossing over it involves non-homologous segments and so has been referred to as "illegitimate" crossing over. Two theories have been advanced in explanation of how translocations are produced. According to one theory (advanced by Serebrovsky) two chromosomes first touch and cross (Fig. 104a). Breakage then occurs at the point of contact

a THE "CONTACT FIRST" THEORY

b THE "BREAKAGE FIRST" THEORY
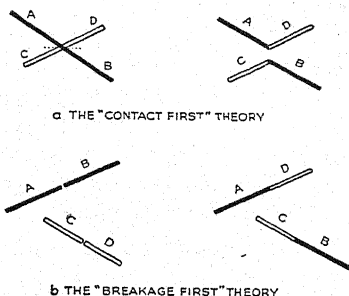
Fig. 104. Theories of the mechanism of crossing over.

(possibly as the result of X-ray treatment), and the segments then unite in a new arrangement (A with D and B with C in Fig. 104a). According to the second theory (the one which we have been assuming), when a translocation takes place two chromosomes are independently broken into two (without first crossing), and the fragments then happen to come together in a new arrangement (Fig. 104b). On the first theory contact occurs first, then breakage. On the second theory breakage occurs first and then contact. It has been shown by Muller that the second theory (the "breakage first" theory) is the correct one, as follows.

Suppose that two chromosomes (AB and CD) should come into contact and cross each other as assumed on the first theory. Then if X-rays should strike the two chromosomes at the point of contact, one "hit" would suffice in breaking both, just as one stroke of a knife might cut two crossed ropes. Now, if only one hit were necessary per translocation, then doubling the dosage of the X-rays would double the number of hits and therefore double the number of translocations; three times the dosage would produce three times the number of translocations. In general, the per cent of translocations would be directly proportional to the dosage; that

is, the per cent would be a simple "linear function" of the dosage. But suppose that two chromosomes did not first cross and make contact. Then it would take a separate hit to break each one into two and the per cent of translocations would not be a linear function of the dosage. Suppose, for example, that with a given dosage one cell in 10 contained a break in chromosome AB and one in 10 a break in CD. Then we could regard the $\frac{1}{10}$ that contained a break in AB as a random sample and $\frac{1}{10}$ of this sample would contain a break in CD. In other words, only $\frac{1}{10} \times \frac{1}{10}$ or $\frac{1}{100}$ of the sample would contain a break in both AB and CD at the same time. If the dosage were doubled, the figure would be $\frac{2}{10} \times \frac{2}{10}$ or $\frac{4}{100}$; with three times the dosage it would be $\frac{3}{10} \times \frac{3}{10}$ or $\frac{9}{100}$. In general, the per cent of translocations would be proportional to the *square* of the dosage (1, 4, 9 over 100 with dosages of 1, 2, 3 respectively). It has been found experimentally that this is the case. Hence it follows that it takes two independent hits to produce a translocation. The same rule applies in general to chromosomal rearrangements which involve two breaks (though very small deletions require only one hit).

When chromosomes are broken into segments, as by X-rays, the broken ends might become reunited immediately. This would not permit the segments to accumulate in the cell and come together in new arrangements. In the case of the egg cells of Drosophila the segments come together very soon after breakage, and thus not many chromosomal rearrangements are produced in the egg cell, even with relatively high dosage of X-rays. But in the case of the sperm cells the broken ends do not unite until fertilization, and the fragments continue to accumulate as long as X-ray treatment is given. Thus a fairly large number of fragments might accumulate and when fertilization takes place, there is a good chance that some of the fragments will come together in the egg cytoplasm in new arrangements. As a result, X-rays of a given dose produce a much higher number of chromosomal rearrangements when applied to sperm cells than when applied to egg cells. The coming together of the broken ends is a purely accidental matter, and the per cent of rearrangements requiring two breaks is usually proportional to the square of the dosage, as above explained.

Normal crossing over (in untreated material) probably takes place in very much the same way as in the abnormal cases just

considered with the exception that breakage is caused by a strain and occurs at corresponding loci in a given pair of chromosomes. The crossing of the strands seen in a chiasma is not the cause of crossing over but the result of it. The strands first break, the broken ends then cross over and connect, thus forming a chiasma.

SUMMARY

1. When crossing over takes place, there is an orderly interchange of chromosome segments. Only corresponding segments are interchanged. The linear order of the genes is not changed, neither are any genes lost or transferred to unlike chromosomes. But as a comparatively rare event in nature, chromosomes sometimes become broken up in an irregular manner, and the segments then become united in an irregular manner. As a result the genes undergo irregular rearrangements. X-rays readily break up chromosomes, and therefore they readily cause irregularities of all sorts in the arrangements of the genes.

2. Sometimes non-homologous chromosome segments become interchanged, as when $AB\ CD$ give rise to $AD\ CB$ by an interchange of B and D. Such a segmental interchange is known as *translocation*.

3. The combination of two normal chromosomes ($AB\ CD$) and two translocated ($AD\ CB$) gives rise to a translocation heterozygote $\left(\dfrac{AB\ CD}{AD\ CB}\right)$.

4. In Drosophila, homologous chromosome segments pair throughout their length in a translocation heterozygote at the reduction division. In a heterozygote of composition $\dfrac{AB\ CD}{AD\ CB}$, the chromosomes form a four-armed figure, each arm consisting of two homologous segments (AA, BB, CC, and DD). The segments then separate over most of their length but remain together at their ends for a while, forming a ring, thus:

$$\begin{array}{l} A\ B{\rule[0.5ex]{1.5em}{0.4pt}}B\ C \\ |\qquad\qquad\quad | \\ A\ D{\rule[0.5ex]{1.5em}{0.4pt}}D\ C \end{array}.$$

Sometimes adjacent chromosomes go to the same pole (as when $AB\ BC$ go to one pole and $AD\ DC$ to the other). The resultant gametes contain duplications and deficiencies and are "unbalanced." But sometimes the ring first twists into an 8, and then alternate chromosomes go to the same pole ($AB\ CD$ to one pole, $AD\ CB$ to the other), forming "balanced" gametes.

5. In a translocation heterozygote of composition $\dfrac{AB\ CD}{AD\ CB}$, there are only two balanced classes of gametes: $AB\ CD$ and $AD\ CB$. These are the only two classes which give rise to viable offspring when the heterozygote is bred to a normal $\left(\dfrac{AB\ CD}{AB\ CD}\right)$.

6. It is possible to detect translocations with the aid of mutant genes in the chromosomes involved. Thus, in Drosophila vestigial (vg) is in the "second" chromosome, and spineless (ss, absence of the larger bristles) in the "third," each of these mutants being recessive to its normal allele (for long wings and for not-spineless). Normal males might be treated with X-rays and mated to vestigial spineless females $\left(\dfrac{+\ +}{+\ +}\ \sigma^{\!\!\!7} \times \dfrac{vg\ ss}{vg\ ss}\ \circ\!\!\!\!\varphi\right)$.

The F_1 males would be of genotype $\dfrac{vg\ ss}{+\ +}$. Ordinarily, these would produce four classes of gametes ($+\ +$, $vg\ ss$, $vg\ +$, and $+\ ss$). But if the treatment produced translocations between the second and third chromosomes, then some of the F_1 males would receive the translocated chromosomes ($+\ +$). They would be translocation heterozygotes, and they would produce only two classes of balanced gametes: $+\ +$ and $vg\ ss$. Hence if the F_1 males were bred to vestigial spineless females $\left(\dfrac{vg\ ss}{+\ +}\ \sigma^{\!\!\!7} \times \dfrac{vg\ ss}{vg\ ss}\ \circ\!\!\!\!\varphi\right)$, those with translocations would produce only two viable classes of offspring $\left(\dfrac{+\ +}{vg\ ss}\ \text{and}\ \dfrac{vg\ ss}{vg\ ss}\right)$. The absence of the other two expected classes (the recombination classes) would tell us that they contained a translocation between the second and third chromosome.

7. In a translocation heterozygote of genotype $\dfrac{vg\ ss}{+\ +}$, vg and ss are in effect linked, since the recombination classes are not produced in the test cross. In general, when two chromosomes are involved in a translocation, their genes behave as though they were linked, in a test cross.

8. Two such unbalanced gametes as $AB\ CB$ and $AD\ CD$ are said to be "complementary duplication-deficiencies" because each has in extra amount what the other lacks. Their combination would produce $\dfrac{AB\ CB}{AD\ CD}$. This offspring would be viable because it contains $AB\ CD$ (diagonally opposite in the formula) and $AD\ CB$ (also diagonally opposite) and is the equivalent of a translocation heterozygote $\left(\dfrac{AB\ CD}{AD\ CB}\right)$.

9. It can be shown that in Drosophila a translocation heterozygote produces unbalanced gametes (as well as balanced). For when two heterozygotes are bred together $\left(\dfrac{AB\ CD}{AD\ CB} \times \dfrac{AB\ CD}{AD\ CB}\right)$, complementary duplication-deficiency gametes combine and produce viable offspring, as when $AB\ CB$ and $AD\ CD$ combine to produce $\dfrac{AB\ CB}{AD\ CD}$ (in which diagonally opposite chromosomes are balanced). This can be proved by a mating of

two translocation heterozygotes each of composition $\dfrac{AB}{AD}\dfrac{CD}{CB}$ and in which each B segment of one parent contains vg and each D segment of the other parent ss, thus: $\dfrac{AB(vg)}{AD}\dfrac{CD}{CB(vg)} \times \dfrac{AB}{AD(ss)}\dfrac{CD(ss)}{CB}$. The combination of $AB(vg)\ CB(vg)$ and $AD(ss)\ CD(ss)$ would then produce $\dfrac{AB(vg)}{AD(ss)}\dfrac{CB(vg)}{CD(ss)}$. Such offspring appear vestigial spineless and they tell us that the heterozygous parents produce the two complementary duplication-deficiency gametes in question.

10. In corn pollen cells with deficiencies and duplications are shriveled up and sterile. The same sort of thing applies to the megaspores (the cells which give rise to the eggs). Hence a translocation heterozygote produces only the balanced gametes in corn.

11. Genic balance is necessary for growth. In animals the mature sperm and egg cells do not undergo any growth before fertilization, and hence they do not necessarily degenerate if they are genically unbalanced. But pollen cells and megaspores must grow before they can produce gametes, and hence they are sterile if they are genically unbalanced.

12. It is possible to locate the breakage points in a translocation heterozygote by means of linkage experiments. Thus if $ABCD$ were originally in one chromosome and $EFGH$ in another, then if a translocation occurred and a test cross were made, offspring containing $abGH$ (or $ABcd$) and $dcFE$ (or $DCba$) would tell us that the break in chromosome $ABCD$ was to the right of B and to the left of C (see p. 260, Fig. 86).

13. In Datura, translocations have occurred in nature and have produced changes in the segmental arrangement of the chromosomes, as from $1\cdot2\ 3\cdot4$ to $1\cdot4\ 3\cdot2$. Races homozygous for a given arrangement $\left(\text{as } \dfrac{1\cdot2}{1\cdot2}\dfrac{3\cdot4}{3\cdot4} \text{ and } \dfrac{1\cdot4}{1\cdot4}\dfrac{3\cdot2}{3\cdot2}\right)$ are called "prime types." These do not differ from one another in outward appearance. But if two plants do not happen to belong to the same prime type and if they are crossed $\left(\text{as } \dfrac{1\cdot2}{1\cdot2}\dfrac{3\cdot4}{3\cdot4} \times \dfrac{1\cdot4}{1\cdot4}\dfrac{3\cdot2}{3\cdot2}\right)$, they then produce a translocation heterozygote in the F_1 $\left(\text{as } \dfrac{1\cdot2}{1\cdot4}\dfrac{3\cdot4}{3\cdot2}\right)$, and in such a heterozygote, the chromosomes form a ring at synapsis, such as $\begin{vmatrix} 1\cdot2 - 2\cdot3 \\ 1\cdot4 - 4\cdot3 \end{vmatrix}$. Translocations have caused segmental rearrangements in other species besides Datura, but as a rule they have not caused differences in the outward appearance of plants or animals.

14. Sometimes a chromosome gets broken into three segments, and the

middle segment gets turned round, as when $A \cdot B \; C \cdot D$ gives rise to $A \cdot C \; B \cdot D$. This turning around of a middle segment is called an *inversion*.

15. The combination of two gametes, one containing an inversion $(A \cdot C \; B \cdot D)$ and the other normal $(A \cdot B \; C \cdot D)$, produces an offspring heterozygous for the inversion $\left(\dfrac{A \cdot C \; B \cdot D}{A \cdot B \; C \cdot D}\right)$. This is called an "inversion heterozygote." The offspring might be heterozygous for both the inversion and the recessive genes $a \cdot b \; c \cdot d$, thus: $\dfrac{A \cdot C \; B \cdot D}{a \cdot b \; c \cdot d}$.

16. In an inversion heterozygote the synapsis of the normal and the inverted chromosome leads to the formation of a loop.

17. In an inversion heterozygote, the single crossovers that occur within the limits of the inversion contain either two centromeres or none, and in the case of a female Drosophila, they get lost in the polar bodies. Hence in effect crossing over is suppressed within the limits of an inversion in an inversion heterozygote. Crossover "suppressors" are usually inversions.

18. In Drosophila one inversion might be followed by a second in the same chromosome, giving a "compound" inversion. Sometimes the second "overlaps" the first. At synapsis, the normal chromosome and the simple inversion form a simple loop; but normal and overlapping inversions form a compound loop. The simple inversion and the overlapping would form a simple loop, since they differ by only one inversion.

19. If race A is normal and C differs from it by two overlapping inversions, it is not likely that A gave rise to C in one step but in two (A to B and B to C, stage B differing from A and C by only a simple inversion). It is thus possible to trace the evolutionary relationships of inversion races, as, for example, in *Drosophila pseudo-obscura*.

20. Sometimes a chromosome becomes broken up into three segments, and the middle segment gets lost. The loss of a segment from a chromosome is called a *deletion*.

21. Large deletions as a rule act as lethals. But small deletions are not always lethal.

22. In Drosophila, Notch wings are caused by a small deletion in a certain part of the X chromosome, usually not more than 1.5 map units in length. The Notch deletion acts as a lethal in males or when homozygous in females, so that Notch flies are always heterozygous females.

23. In Drosophila, very small deletions, known as "Minutes," cause a reduction in the size of the bristles. Over seventy-five "Minutes" have been found, distributed in all four chromosomes of Drosophila.

24. It is possible that in Drosophila visible mutations, such as yellow body, might often be caused by deletions that are too small to be visible under the microscope. But it does not seem likely that mutations are always losses or mere rearrangements of the chromatin.

25. In Drosophila a translocation between the X and the Y chromosome might result in an X with an arm of the Y chromosome attached to it. A second translocation between the attached arm and an X might then result in attached X's.

26. A "duplication" is a segment of a chromosome present in extra amount in a cell, as compared to the normal. Thus if a cell normally contained a pair of chromosomes each with an A segment $\left(\text{as in } \dfrac{ABC}{ABC}\right)$, a third A would be a duplication.

27. In Drosophila a sperm cell might contain an X from which a large segment has been deleted, leaving only a small terminal segment of active chromatin. This sperm cell might fertilize an attached-X egg. In the resulting female, the terminal segment of the X would be represented three times—twice in the attached X's and once in the deleted X, and the deleted X would therefore be a duplication. Attached-X eggs cannot develop if they contain a large duplicated segment of the X, but if the duplication is small, they might, in which event they often develop some abnormality such as small bristles or rough eyes.

28. Sometimes one small segment of a chromosome is followed by another one exactly like the first. This form of a duplication is known as a "repeat," and might be caused by unequal crossing over.

29. In Drosophila, the mutant "Bar" contains a repeat.

30. When a chromosome is broken into three segments, the ends of the middle segment might come together and form a ring chromosome.

31. In Drosophila, one attached X might get broken near its attached end and the other near its unattached end. The two broken ends might then join to form a ring X chromosome, usually referred to as "closed X" (X^c).

32. Ring chromosomes do not occur in nature. They tend to get lost at the reduction division as the result of crossing over, for they then form a dicentric ring of double size and get caught in the polar bodies.

33. Sometimes inversions and translocations have a visible effect on development, the adult having some mutant trait (such as wings with rough margins). The mutant trait is due entirely to the changed positions of genes relative to one another, not to any change in a gene itself. The visible change due to a changed arrangement of genes is called the *position effect*. A duplication might have a position effect, as in Bar eyes.

34. Translocations and inversions involve first the breakage of chromosomes and then the union of the broken ends, according to the "breakage-first" theory. The chromosomes do not first cross each other and then break at the point of contact. The "breakage-first" theory is supported by X-ray experiments which show that the per cent of translocations produced by X-rays is proportional to the square of the dosage.

PROBLEMS

1. In Drosophila Curly wings (Cy) is a dominant mutation in the second chromosome, and Dichaete (D, absence of all the larger bristles except two pairs, and spread-apart wings) is a dominant in the third chromosome. Given the cross $\dfrac{Cy\ D}{+\ +}\ \sigma \times \dfrac{+\ +}{+\ +}\ \varphi$. Tell what four classes of gametes each parent would form and what four genotypic classes of offspring would therefore be produced by the cross. Give the phenotypes of the off-spring (name the $+\ +$ offspring "normal" and the rest after their mutant traits, as Curly for $Cy\ +$).

2. Given a male of genotype $\dfrac{1(Cy)\cdot2\ \ 3(D)\cdot4}{1(+)\cdot4\ \ 3(+)\cdot2}$ in which $1\cdot2$ and $3\cdot4$ are chromosomes with the normal segmental arrangement and $1\cdot4$ $3\cdot2$ are translocated, Cy and D being in segments 1 and 3, respectively, of the un-translocated chromosomes. Tell what classes of gametes this male would produce and tell which ones would contain neither a deficiency nor a duplication.

3. a. Assume that the above male was crossed to a wild type female, thus $\dfrac{1(Cy)\cdot2\ \ 3(D)\cdot4}{1(+)\cdot4\ \ 3(+)\cdot2}\ \sigma \times \dfrac{1(+)\cdot2\ \ 3(+)\cdot4}{1(+)\cdot2\ \ 3(+)\cdot4}\ \varphi$. Then give the genotypes and phenotypes of the viable offspring (those capable of development to the adult stage). Tell also which offspring are inviable and why.

b. Suppose that the translocated chromosomes in the male parent in Problem 3a had been derived from his father, a wild type male. To what was this father then bred in order to produce the heterozygous male in question?

4. Suppose you X-rayed a wild type male and produced a translocation in a few of his sperm cells, between the second and third chromosomes. Tell how you would go about detecting these translocations with the aid of $Cy\ D$ stock.

5. In Drosophila pink (p) is an eye-color mutation in the third chromosome recessive to red ($+$), and bent (bt) is a wing mutation in the fourth chromosome recessive to straight ($+$). Suppose you X-rayed some wild males and produced some translocations between the third and fourth chromosomes. Tell how you would go about detecting the translocations with the aid of pink bent stock.

6. Mutant genes used to identify or "mark" chromosomes in crosses are known as "markers" (as when Cy and D are used to mark the second and third chromosomes, or pink and bent the third and fourth, in the above crosses). In general, tell how you would detect a translocation between any two chromosomes with the aid of markers, in Drosophila.

7. Given two chromosomes with the dominants *ABCDEF* and *GHIJKL*. Assume that a break takes place between *CD* and between *IJ* and that the segments to the right of each break become interchanged. Give the resulting two translocated chromosomes.

8. Assume that a fly is heterozygous for the above two translocated chromosomes and for two untranslocated containing the recessives *abcdef* and *ghijkl*. Make a diagram of the two untranslocated chromosomes (with the recessives) above and of the two translocated (with the dominants) below. Next show how these chromosomes pair at the reduction division in the heterozygote. Indicate the breakage points by short cross lines.

9. Assume that crossing over took place between locus *C* and the breakage point in the above heterozygote. Then give the composition of the two resulting crossover classes of gametes (assuming there had been no crossing over other than that mentioned).

10. If you were given either of the above two crossovers what conclusion could you draw from them alone as regards the location of the breakage point? What two crossovers would tell you that the breakage point was to the left of locus *d*? Between what two loci would the breakage point be located by the crossovers first considered and those now?

Note. Problems 11–16 form a series dealing with the location of the breakage points in translocation heterozygotes. The data are selected from Dobzhansky.

11. In Drosophila the fourth chromosome (very small) contains eyeless (*ey*), a mutant gene which is recessive to its normal allele (+ or non-eyeless). The third chromosome (very long) contains the following mutant genes, all recessive (except Dichaete) to their normal alleles:

roughoid (*ru*), rough eye surface
hairy (*h*), hairs along the wing veins
Dichaete (*D*), previously described
thread (*th*), unbranched aristae (feelers attached to the antennae)
scarlet (*st*), bright red eye color
curled (*cu*), wings curved upwards
stripe (*sr*), dark longitudinal stripe on the thorax
sooty (*e^s*), dark body color (an allele of ebony)
claret (*ca*), purplish pink eye color.

Given a pair of fourth chromosomes (very small) and a pair of third (large), having the following distribution of "markers," and arbitrarily subdivided into segments as indicated by the numbers:

$$\frac{ey}{1\,|2} \quad \frac{ru\ h\ +\ th}{3} \,|\, \frac{st\ cu\ sr\ e^s\ ca}{4}$$

$$\frac{+}{1\,|2} \quad \frac{+\ +\ D\ +}{3} \,|\, \frac{+\ +\ +\ +\ +}{4}$$

Assume that segments (2) and (4) of the lower two chromosomes become interchanged but that the upper two remain normal. Then draw the chromosomes of a fly heterozygous for the two normal and the two translocated (before synapsis) and show next how these chromosomes come together at synapsis.

12. If the above heterozygote were a male (in which there is no crossing over) and if it were crossed to a female pure for the untranslocated chromosomes, then tell what classes of gametes it would form capable of giving rise to viable offspring. (Use only the segment numbers to designate the chromosomes.) In these gametes, which third chromosome would always be associated with the fourth chromosome containing (1) eyeless (*ey*), and (2) non-eyeless (+)?

13. Assume that the above heterozygote were a female (in which there is crossing over) and that you were attempting to determine the breakage point in the third chromosome from an examination of the crossover classes essentially as done in principle in Problem 9. Then tell (1) which crossover class of gametes which contains eyeless would tell us that the break in the third chromosome was to the right of thread (*th*), (2) which non-eyeless crossover class would tell us the same thing. Tell also which two crossover classes (with eyeless and with non-eyeless) would tell us that the break was to the left of scarlet (*st*). (Use synapsis diagram made for Problem 11.)

14. Tell to what kind of a male you would cross the above heterozygous female in order to detect the crossover classes of gametes referred to in Problem 13, and tell what the appearance of the resulting crossover classes would be.

15. A female Drosophila is heterozygous for a translocation between the fourth and third chromosomes. She contains the markers shown in Problem 11 and the translocated chromosomes are the two lower shown in Problem 11 (the non-eyeless and *D*-chromosomes), but the break is at a different place in the *D* chromosome than shown in Problem 11. This female, when test crossed, produces numerous classes of offspring, including the two following crossover classes: (1) $ey + + D + + cu\ sr\ e^s\ ca$ and (2) $ey\ ru\ h + th\ st + + + +$. Give the location of the breakage point. Tell which two non-eyeless crossover classes would also have served to locate the breakage point.

16. Suppose the translocated chromosomes in the heterozygous female in Problem 15 had been derived from her father, a non-eyeless Dichaete male treated with X-rays. To what was this father mated in order to produce the heterozygous female in question?

17. Outline an experiment for detecting the breakage points produced by X-rays in the third chromosome of Drosophila, when the breakages result in translocations between the third and fourth chromosomes. Assume that we begin by treating non-eyeless Dichaete males, and that we employ the markers given in Problem 11.

18. In corn a translocation heterozygote produces about 50 per cent genically unbalanced pollen cells, but these are sterile (shriveled and incapable of growth down the pistils), and translocation heterozygotes in corn are therefore referred to as "semi-sterile." Given chromosomes $1 \cdot 2$ $3 \cdot 4$ in corn. Assume that segments 2 and 4 become interchanged, producing the translocated chromosomes $1 \cdot 4$ $3 \cdot 2$, and that the translocation heterozygote is then got $\left(\dfrac{1 \cdot 2 \; 3 \cdot 4}{1 \cdot 4 \; 3 \cdot 2} \right)$. Assume further that the heterozygote is crossed to a normal $\left(\dfrac{1 \cdot 2 \; 3 \cdot 4}{1 \cdot 4 \; 3 \cdot 2} \times \dfrac{1 \cdot 2 \; 3 \cdot 4}{1 \cdot 2 \; 3 \cdot 4} \right)$. Then give the chromosome formulas of the two classes of offspring that are produced. Tell which class is semi-sterile and what proportion it forms of the total offspring.

19. Suppose a semi-sterile corn plant were crossed to a normal and produced 50 per cent semi-sterile and 50 per cent normal offspring. What would you suspect to be the cause of the semi-sterility?

20. Make a diagram to show how the chromosome segments pair in a corn translocation heterozygote of composition $\dfrac{1 \cdot 2 \; 3 \cdot 4}{1 \cdot 4 \; 3 \cdot 2}$. Put the gene a in about the middle of segment 1 of chromosome $1 \cdot 2$ and the dominant allele A at the corresponding locus in $1 \cdot 4$. Assume that there is no crossing over between the arms (a always remaining in $1 \cdot 2$ and A in $1 \cdot 4$). Assume further that we cross this heterozygote to a pure normal (chromosomes untranslocated) of genotype a/a. Then tell what classes of offspring will be produced with regard to the a locus and tell which class will be semi-sterile.

21. Assume that in the above cross there is 20 per cent of crossing over between the a locus and the breakage point in the translocation heterozygote. Then classify the crossover offspring with regard to both the a locus and semi-sterility, and tell what per cent each crossover class is of the total offspring. Tell also what per cent each non-crossover class is of the total, and give the phenotype of each.

22. Give the distance between a and the breakage point in the above semi-sterile corn plant.
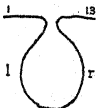
23. Assume that a given chromosome is divided into three segments, thus: $a \, b \cdot c \, d \, e \, f \cdot g \, h$ and that the middle segment is inverted, giving $a \, b \cdot f \, e \, d \, c \cdot g \, h$. Assume further that an inversion heterozygote is got of genotype $\dfrac{a \; b \cdot f \; e \; d \; c \cdot g \; h}{A \; B \cdot C \; D \; E \; F \cdot G \; H}$. Make a diagram to show how the chromosomes pair in this heterozygote.
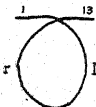
24. Assume that crossing over took place between loci d and e in the above heterozygote. Give the resulting crossovers and tell what each is a duplication for and a deficiency for. Do the same for crossing over between c and d.

25. Assume that the centromere is at the right end of the chromosome in the above heterozygote (the end with h or H). Tell why one of the crossover products always gets both centromeres and the other neither, when crossing over takes place within the limits of the inversion. Accordingly, what would always happen to these crossovers at the completion of the reduction division? Would this apply to the crossovers outside the limits of the inversion (between a and b, or between g and h). Why or why not?

26. How could you determine, from the offspring produced by a test cross of the above heterozygote, that section c to f had been inverted?

27. In diagrams a, b, below, the segment between the single lines includes one inversion and the segment between the double lines includes a second inversion in a chromosome carrying the first one. Show how the chromosome with the two inversions would pair with a normal chromosome in the heterozygote. To do this follow the method shown in Fig. 92 on p. 268. First consider just the normal chromosome by itself, and bring together the first two breakage points and then the second two. Next derive the chromosome with the double inversion and put it beside

the normal chromosome. Draw the first loop either thus

or thus , depending on whether the second loop is to be connected with the right half (r) or left half (l) of the first one.

a. $\underline{1 \quad 2} \mid \underline{3 \quad 4 \quad 5 \quad 6} \mid\mid \underline{7 \quad 8} \mid \underline{9 \quad 10 \quad 11} \mid\mid \underline{12 \quad 13}$

b. $\underline{1 \quad 2} \mid \underline{3 \quad 4} \mid\mid \underline{5 \quad 6 \quad 7 \quad 8} \mid \underline{9 \quad 10 \quad 11} \mid\mid \underline{12 \quad 13}$

28. Given a Drosophila female which carries one normal X and one with a deficiency extending from the locus of white eyes (w) to the locus of facet (fa, rough eyes), the deficiency causing the female to have "Notch" wings. To what might you cross the Notch female to show that she carried this deficiency and what kind of offspring (females and males) would she produce in evidence of the deficiency?

14. *CYTOGENETIC MAPS*

A CHROMOSOME map does not necessarily tell us the actual locations of the genes in a chromosome, for the map is based entirely on per cents of crossing over, not on a direct examination of the physical chromosome under the microscope. It is a *crossover* map. But it is possible to determine the actual location of genes. This might be done by first removing a small segment of a chromosome by means of X-rays. We might then examine the chromosome under the microscope to see what segment had been removed. Next we might determine which genes had been removed, by means of linkage tests. In this way we could show that certain genes corresponded with certain segments, and so we could locate the genes within the physical chromosome. A map which shows the actual location of genes in a chromosome involves not only the microscopic examination of the chromosome (or cytological examination), but also genetic analysis in the form of linkage tests. Such a map is referred to as a *cytogenetic* map.

Cytogenetic Maps of Metaphase Chromosomes.—Chromosomes vary greatly in size and shape at different stages of cell division. At metaphase they are usually rod-shaped and are referred to as "metaphase" chromosomes. The first cytogenetic maps showed the location of genes in metaphase chromosomes. They were constructed by Muller and his co-workers.

Figure 105 shows a cytogenetic map of the X chromosome of Drosophila as seen at metaphase, constructed by Muller and Painter. The black portion of the map indicates the physical part of the X chromosome which carries all the sex-linked genes with the exception of bobbed bristles (*bb*). The stippled area indicates the region of the X which is "inert" in the sense that it carries no known genes except bobbed bristles (*bb*). The letters (A, B, C, etc.) below the map indicate the places where the physical chromo-

some was broken in making the map. For example, the segment of the X to the left of A was broken off (by X-rays) and transferred to the "fourth" chromosome. The size of the transferred piece was easily recognized by the fact that it caused a conspicuous increase in the size of the fourth chromosome, which ordinarily is very small (dot-shaped). Parallel to this observation it was found
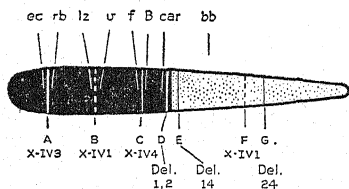
Fig. 105. A cytogenetic map of the X-chromosome of Drosophila, based on the metaphase chromosome. (From Muller in *Zeit. f. ind. Abst. u. Ver.*)

by linkage tests that the gene *ec* (echinus or rough eyes) was no longer linked to the other genes in the X but instead was linked to the genes in the fourth chromosome, and it was therefore concluded that the gene *ec* was in the physical section of the X to the left of break A.

In a second experiment the section of the X between B and F was removed and transferred to the fourth; and correspondingly, it was found that genes *v f B car bb* were no longer linked to the other genes in the X but to those in the fourth chromosome.

An illuminating experiment was one that involved the break C. In this case again, the segment of the X to the left of C was transferred to the fourth chromosome, but although the segment in question was less than half the total length of the X, yet the linkage tests showed that it contained almost all the genes in the X—all up to and including f (forked bristles), which is at point 56 in the chromosome map, the total map length of the X being 66. Hence the right half of the X contains only a few genes. Further tests, similar to those above described, showed that the segment of the X to the left of break D contained all the genes in the X except *bb* (bobbed bristles). In other words, the section of the X stippled in Fig. 105 is practically "inert."

Salivary Gland Chromosomes.—In Drosophila, metaphase chromosomes are extremely small, even when examined under the highest powers of the microscope, and this makes them very unfavorable for detailed study. But it so happens that the chromosomes in the salivary glands of the larva are of enormous size as compared with those in other parts of the animal, and Painter has more recently employed salivary gland chromosomes for the con-

struction of cytogenetic maps. Before discussing the maps themselves, it might be well to say a word about salivary gland chromosomes.

RIGHT ARM OF CHROMOSOME III

X CHROMOSOME

CHROMOSOME IV

CHROMOSOMES FROM OVARIAN TISSUE
(Same enlargement)

CHROMOCENTER

LEFT ARM OF CHROMOSOME III

LEFT ARM OF CHROMOSOME II

RIGHT ARM OF CHROMOSOME II
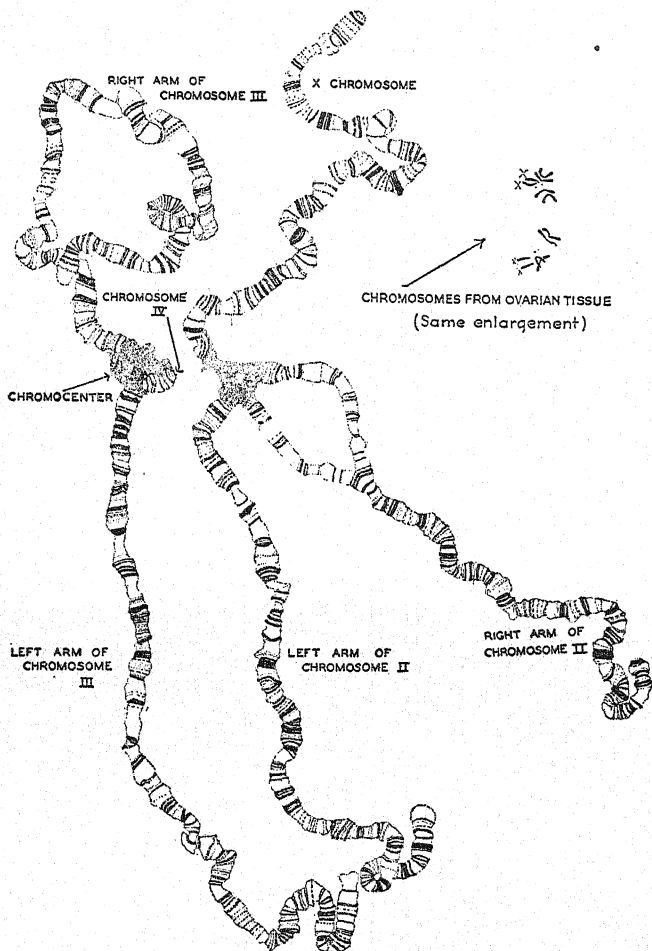
FIG. 106. The salivary gland chromosomes of Drosophila. (From Painter in *The Journal of Heredity.*)

The nucleus is the constructive center of a cell and it is unusually large when big demands are made on it. An insect larva is constantly eating and its salivary glands are constantly building up digestive juices. Hence the nuclei of the salivary gland cells are

Fig. 107. A small segment of a salivary gland chromosome, highly magnified. (From C. W. Metz in *The Journal of Heredity*.)

unusually large. This large size comes about through increase in the size of their chromosomes.

Figure 106 is a drawing of the chromosomes in a salivary gland cell. At the upper right some ordinary chromosomes are drawn to scale. The enormous size of the salivary gland chromosomes at once becomes evident. Another striking feature about these giant chromosomes is their banded structure. The bands differ in ap-

pearance from one small segment of the chromosomes to the next. Some bands are narrow, others broad; some stain deeply, others faintly. Adjacent bands also bunch up into groups of characteristic appearance in a given segment of the chromosome. A group may consist of two broad bands and a narrow one, or of a broad, a narrow, and a broad band, or of a faint band and two dark bands, and so on. For a given kind of chromosome, and for a given segment of that chromosome, the appearance of the bands is uniform from one cell to another, but in going from one kind of chromosome to another in the same cell, or from one segment of a chromosome to another segment, the appearance of the bands differs in a characteristic manner. It is therefore possible from the appearance of the bands to tell with what chromosome and with what particular segment of that chromosome one is dealing. For example, one could, if given just a small segment of the X, identify it from the characteristic appearance of its bands and say that it belongs to the X and to a particular part of the X.

Figure 107 is a photograph of a small segment of a salivary gland chromosome highly magnified. Careful examination of this photograph will show that different segments of the chromosome differ characteristically from one another with regard to the appearance of their bands. Figure 108 is a drawing of the fourth chromosome highly magnified.
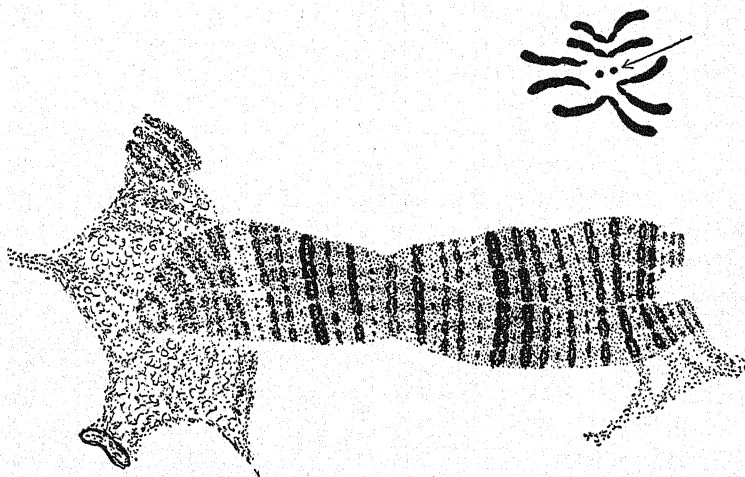
FIG. 108. The fourth chromosome of Drosophila, highly magnified, as seen in the salivary glands of the larva. (From Bridges in *The Journal of Heredity*.)

The size and structure of the salivary gland chromosomes can be accounted for on the basis of their origin. It will be recalled that the important part of a chromosome is a long thin thread— the *chromonema* (Fig. 109a). Along the length of the chromonema are fine granules. Ordinarily the chronomema is very closely wound up and is over a hundred times as long as the envelope which encloses it. In the nucleus of a salivary gland cell the chromonema is not wound up within an envelope; it is unraveled to its full
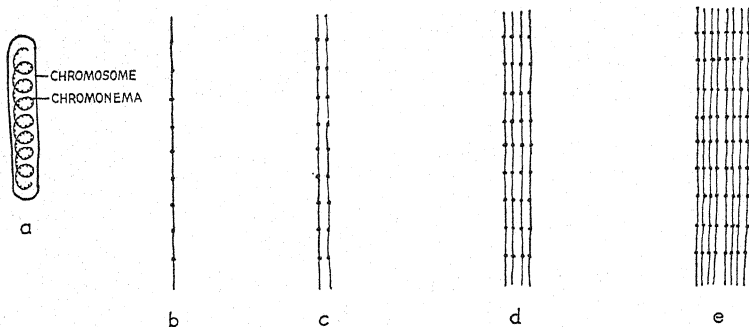
FIG. 109. The origin of the salivary gland chromosomes of Drosophila.

length (Fig. 109b). It is therefore many times as long as an ordinary chromosome—about 150 times as long. Moreover, the chromonemas of the salivary gland cells do a very peculiar thing which ordinarily only chromosomes do in cells that are undergoing the reduction division; namely, homologous chromonemas come together along their length (Fig. 109c). But these do not later separate; they remain permanently paired. Still another peculiar thing then happens. Each thread splits lengthwise into two, as though cell division were about to take place, but the split halves remain together so that there are now four threads in a group, and each thread grows to normal thickness (Fig. 109d). Each of the four threads again splits into two and grows to its normal thickness, giving eight in all. The splitting of the threads continues, followed by growth to normal thickness, and each time this happens the number of threads is doubled. In all there are perhaps ten doublings, resulting in 1,024 threads from each original thread. Thus a giant chromosome is formed, many times the length and thickness of an ordinary chromosome. The bands are due to the

horizontal lining up of granules or other bodies that are structurally differentiated along the length of the individual chromonemas.

The above account of how the salivary gland chromosomes originate is known as the *polytene theory* (or many-thread theory). According to Goldschmidt, however, the paired chromonemas divide only once and then get thicker by growth; they do not divide repeatedly and hence do not become more numerous. The evidence as it now stands favors the polytene theory.

In Fig. 106 the chromosomes radiate out from a small spherical mass of chromatin known as the *chromocenter*.

If the chromosomes in Fig. 106 are counted it will be seen that their number is apparently five. But an ordinary unreduced cell
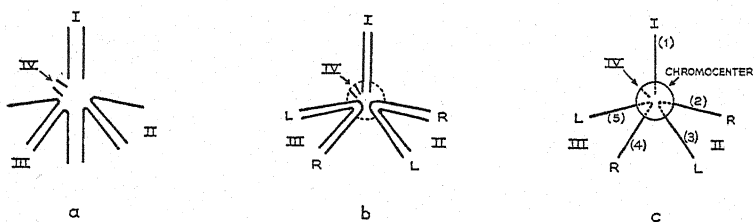
Fig. 110. The chromosome number in the salivary gland cells of Drosophila.

of Drosophila contains four pairs of chromosomes (Fig. 110a). The chromosomes which make up for the four pairs are referred to as the first, second, third, and fourth chromosomes. The first pair consists of two long unbent chromosomes (in the female). These are the *X* chromosomes. The members of the second and third pairs are long and V-shaped, and the fourth pair are very short rods, appearing almost as dots.

How can we derive the chromosomes of a salivary gland cell, apparently five in number, from the four pairs ordinarily seen in an unreduced cell? Each salivary gland chromosome, we just saw, is made up of a pair of chromonemas and their split products. But the two members of the pair are so closely united that they appear to be a single chromosome. Thus the two *X*'s appear to be just one chromosome (Fig. 110b, c). Each pair of *V*'s (the second and third pairs) also forms one chromosome, but the centrally located part of each *V* is imbedded in the chromocenter. Now it is not possible to see the parts of the chromosomes imbedded in the chromocenter as distinct from the chromocenter itself.

Hence the two arms of each of the two V's appear to be separate chromosomes, and this makes them appear to be four chromosomes instead of two. With the X added, we have apparently five chromosomes. The fourth chromosome is not readily visible, because it is almost completely imbedded in the chromocenter. There appear, then, to be a total of five giant chromosomes in a salivary gland cell.

The Salivary Gland Chromosomes of a Deletion Heterozygote.—Figure 111a is a diagram of part of a salivary gland chromosome in which the segments are numbered $1 \cdot 2 \cdot 3$ and each
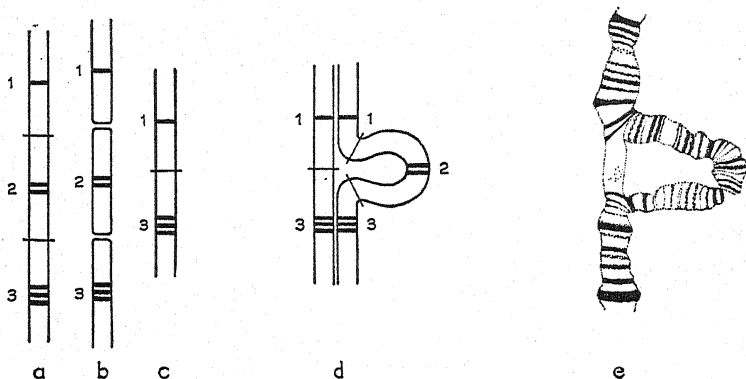
Fɪɢ. 111. The salivary gland chromosomes of a deletion heterozygote. a-d, diagrammatic; e, actual specimen. (Fig. e from Painter in *The Journal of Heredity*.)

segment is shown with a characteristic band arrangement (with one, two, and three bands respectively). If segment 2 were deleted, the band sequence would now be $1 \cdot 3$ (Fig. 111b, c). In a deletion heterozygote, $1 \cdot 2 \cdot 3$ would pair with $1 \cdot 3$ (Fig. 111d). Segment 2 would be buckled, since it has no partner.

Figure 111e is a drawing of the salivary gland chromosomes of a deletion heterozygote as actually seen under the microscope. The buckling of the normal chromosome is plainly shown. The segment which is buckled belongs to the undeleted chromosome, and it corresponds to the segment which is missing in the deleted chromosome. By careful examination of the deleted chromosome, we can tell precisely where the band sequence changes as compared to the normal, and so we can determine precisely what bands have been deleted.

Locating Genes by Means of Deletions.—If a sperm cell with three dominant genes (A B C) fertilized an egg with the three recessive alleles (a b c), then the offspring would ordinarily be A B C/a b c and would not show any of the recessive genes. But suppose that we had first treated A B C with X-rays and deleted the segment of the chromosome containing B, leaving A C. Then the offspring would be $\dfrac{A \quad C}{a\ b\ c}$, and the recessive b would express itself. In this way we should know that the segment containing B had been deleted from A B C. This we should know without having examined the chromosome under the microscope. In other words, we should have determined on purely *genetic* grounds that gene B was missing from chromosome A B C.

We now examine the salivary glands of the heterozygote $\dfrac{A \quad C}{a\ b\ c}$ and we find that a certain segment is buckled. We know that this must correspond to the segment deleted from A C, and that it contains gene b. Hence we know that b must be represented by one or more of the bands in the deleted segment (buckled in the undeleted chromosome). If the deleted segment were large, it would contain many bands, and gene b might be represented by any one or more of these. But if the deleted segment were very small and contained just one band, then the gene would be delimited to the one band. It is for this reason that *small* deletions are preferred in determining what band or bands correspond to a given gene. By means of X-rays, it is possible to produce deletions, some of which might, by accident, remove the dominant allele of some recessive gene. We might also locate genes by means of *overlapping deletions*. Thus, suppose gene K is deleted when, say, segments 21-24 (incl.) are deleted (del. I), and also when segments 24-29 are deleted (del. II). Then K obviously must be in 24—the segment of *overlap*. Two deletions might be very large, but they might overlap by only a very small amount, and thus delimit a gene to that band.

The Salivary Gland Chromosomes of an Inversion Heterozygote.—Figure 112a is a diagram of a salivary gland chromosome in which the segments are in order 1-4. Each segment is shown with a characteristic band arrangement (with one, two, three, and four bands, respectively). An inversion of 2 and 3 would produce the band sequence 1·3·2·4. If the normal and the inverted chromosomes paired (as in the heterozygote), they would

form a loop (Fig. 112c, d). The inversion itself is the result of two breaks, one between segments 1 and 2, the other between 3 and 4.
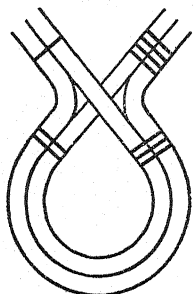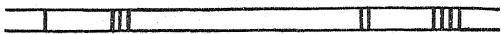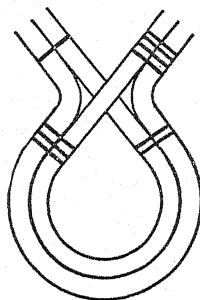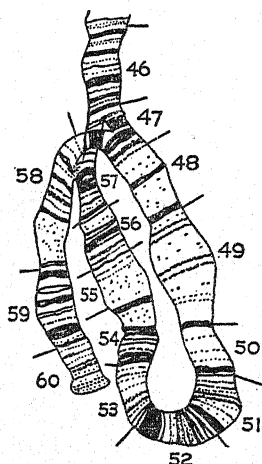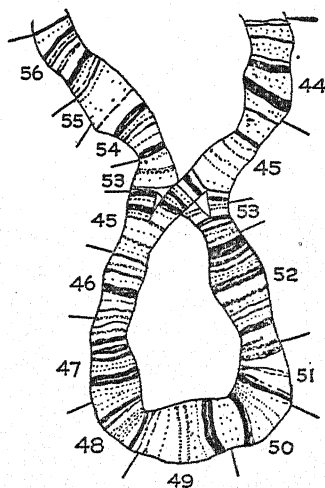
FIG. 112. The salivary gland chromosomes of an inversion heterozygote. In c and c′ the normal chromosome is on the outside of the loop; in d and d′, on the inside. (Fig. c′ and d′ from Dobzhansky and Socolov in *The Journal of Heredity*.)

The salivary glands of the heterozygote would tell us this at a glance, for segments 1 and 4 would be outside of the loop, and 3 and 2 inside.

Figure 112 c′, d′ is a drawing of the salivary gland chromosomes of two inversion heterozygotes as actually seen. Each figure represents the pairing of two chromosomes, one that is normal and one that has an inversion. The two chromosomes are so close together and they are so exactly paired band for band that for the most part they appear like a single chromosome. But at the points where they enter or leave the loop they separate and here it can be seen that they are two. The loop contains two segments side by side: (1) the inverted segment of the chromosome with the inversion and (2) the corresponding segment of the normal chromosome (uninverted).

Locating Genes by Means of Inversions.—In an inversion heterozygote the crossovers within the inversion are suppressed, but those outside are not. Hence linkage tests would tell us whether a given gene was inside or outside the limits of an inversion. In case the gene were outside, the linkage tests would tell us whether the gene was to the left or to the right of the inversion.

A given inversion is of course the result of two breaks (left and right) followed by the inversion of the included segment. If we had two separate inversions (I and II) it would seldom if ever happen that both the left and the right breaks would be in the same places in the two, or even very close together. But it might sometimes happen that the breaks on one side, say, the left, were close together, as indicated by I^l and II^l in Fig. 113. Suppose now that linkage tests
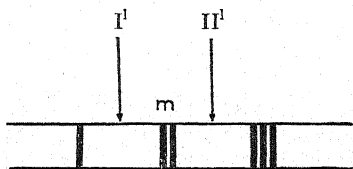
FIG. 113. Locating a gene in a salivary gland chromosome by means of two inversions.

showed that a certain gene (m) was *inside* inversion I but to the *left* of inversion II. Then we should know that m was to the *right* of I^l and to the *left* of II^l. Hence m would be *between* I^l and II^l, and it would be represented by one or more of the bands included between I^l and II^l. Two right inversion breaks might be used equally well in locating a gene provided they are close together.

It is obvious that the closer together two inversion breaks are (as I^l and II^l), the more narrowly can we define the limits within which a gene is located in the salivary gland chromosome. It would be unusual to get two inversions in which either break in the one was close to either break in the other, and to increase the chances of having two such inversions it would be necessary to produce a

large number of inversions. This can be done by means of X-rays.

**Salivary Gland Chromosomes in Translocation Hetero-
zygotes.**—Figure 114a, upper part, shows two salivary gland chromosomes in which the segments are numbered 1·2 and 3·4 (normal), and each segment is shown with a characteristic band arrangement. An interchange of segments 2 and 4 would result in two translocated chromosomes with the band sequence 1·4 3·2 (Fig. 114a, lower). If the normal and translocated chromosomes paired (as in a heterozygote), they would form a four-armed figure (Fig. 114b). Each arm consists of two homologous
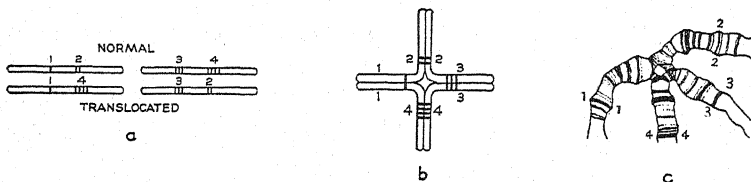
Fig. 114. The salivary gland chromosomes of a translocation heterozygote. a, b, diagrammatic; c, actual specimen. (Fig. c from Mackensen in *The Journal of Heredity*.)

segments (paired). Thus in Fig. 114b arm 1 consists of segment 1 of chromosome 1·2 and of segment 1 of chromosome 1·4. One of the segments in question belongs to a normal chromosome (1·2), the other to a translocated (1·4). The two chromosomes (1·2 and 1·4) correspond band for band within the one arm (section 1), but at the point where they no longer correspond they enter different arms (2 and 4). This marks the exact point at which the breakage occurred in chromosome 1·2 when the translocation took place. In like manner we could determine the exact breakage point in chromosome 3·4.

Figure 114c is a drawing of the salivary gland chromosomes as actually seen in a translocation heterozygote. The chromosomes form a four-armed figure, as above described. Each arm of the figure appears to be a single chromosome, but actually it is made up of a pair of homologous segments, as can plainly be seem at the base of each arm, where the two segments are slightly pulled apart. Thus for example the arm numbered 1 in Fig. 114c consists of the homologous segments of two chromosomes, one of which has a second segment in arm 2, the other in arm 4. One of these

chromosomes is normal (1·2), the other translocated (1·4). At the point where the two chromosomes leave arm 1 and enter arms 2 and 4, respectively, their band sequence no longer corresponds, and this marks the exact breakage point. Previous study will have shown what the normal sequence of the bands is (1·2). Hence we should know at just what point (or between what bands) the breakage occurred with reference to the normal arrangement of the bands.

In Drosophila a very small chromosome (the "fourth") might be involved in a translocation with one of the larger ones (Fig. 115a). In the heterozygote the salivary gland chromosomes
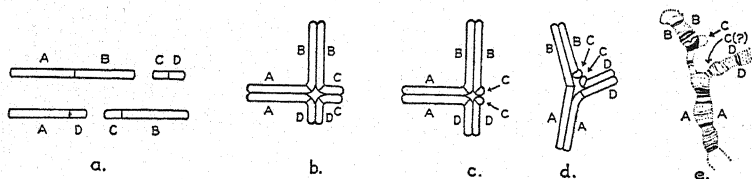
Fig. 115. The salivary gland chromosomes of a translocation heterozygote in which the fourth chromosome is involved. (Fig. e from Painter in *The Journal of Heredity*.)

would form a four-armed figure as usual, but two of the arms would be derived from the short fourth chromosome, and they would be comparatively short (Fig. 115b). If the break in the fourth chromosome had not been in the middle of the chromosome but nearer one end, then one of the segments (say, C) might have been extremely small, and the arm formed by this segment would have been correspondingly small and hardly noticeable (Fig. 115c, d). An actual case of this kind is shown in Fig. 115e.

It is comparatively easy to locate the breakage point in one of the longer chromosomes when it is involved in a translocation with the fourth. For the longer chromosome can be easily identified from the fact that it forms the two longer arms of the four-armed figure in the heterozygote, and the breakage point can then be located at the base of the two longer arms.

Locating Genes by Means of Translocations.—When a chromosome is involved in a translocation, a break occurs between two genes in the *map* of the chromosome, because the chromosome itself is broken into two. It is possible to locate the break in the map by means of linkage tests (p. 259). In this

way, we can determine which genes in the map are to the left of the break and which to the right. A given translocation of course involves breaks in two chromosomes but in locating a gene in a given chromosome we are concerned only with breaks in that chromosome.

Suppose now that we had two translocations and that each involved a break in a given kind of chromosome (say, the second chromosome of Drosophila), as indicated by I and II in Fig. 116. Suppose further that linkage tests told us that a certain gene (p) was to the right of break I and to the left of break II. Then we should know that p was located between I and II and that it must therefore be represented by one or more of the bands included between I and II.

TRANS.   TRANS.
I   II
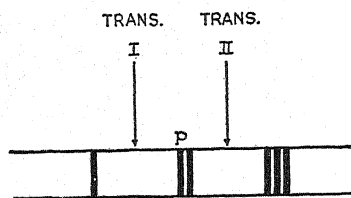
FIG. 116. Locating a gene in a salivary gland chromosome by means of two translocations.

The closer two translocation breaks are together, the more precisely can a gene be located by means of them. Large numbers of translocations are necessary in order to get two which happen to involve close breaks in a given kind of chromosome, but translocations can be produced at will by means of X-rays and accidentally two are sometimes got which involve close breaks.

The Construction of Cytogenetic Maps in Summary.—In summary, the genes on a chromosome map can be correlated with the bands of a salivary gland chromosome by means of deletions, inversions, and translocations. Of these three types of chromosomal alterations, deletions are the most useful for making the correlation in question, particularly minute deletions, for these make it readily possible to delimit the gene to a very few bands.

The principle involved in correlating genes with bands of salivary chromosomes is the same whether deletions, inversions, or translocations are used in making the correlation. In all cases when certain bands are removed from their normal positions in a salivary chromosome, certain genes are also removed, as shown by linkage tests. Hence bands and genes correspond.

Cytogenetic and Chromosome Maps Compared.—Figure 117, top, shows a cytogenetic map of the X chromosome of Drosophila with a chromosome map above for comparison. It will be
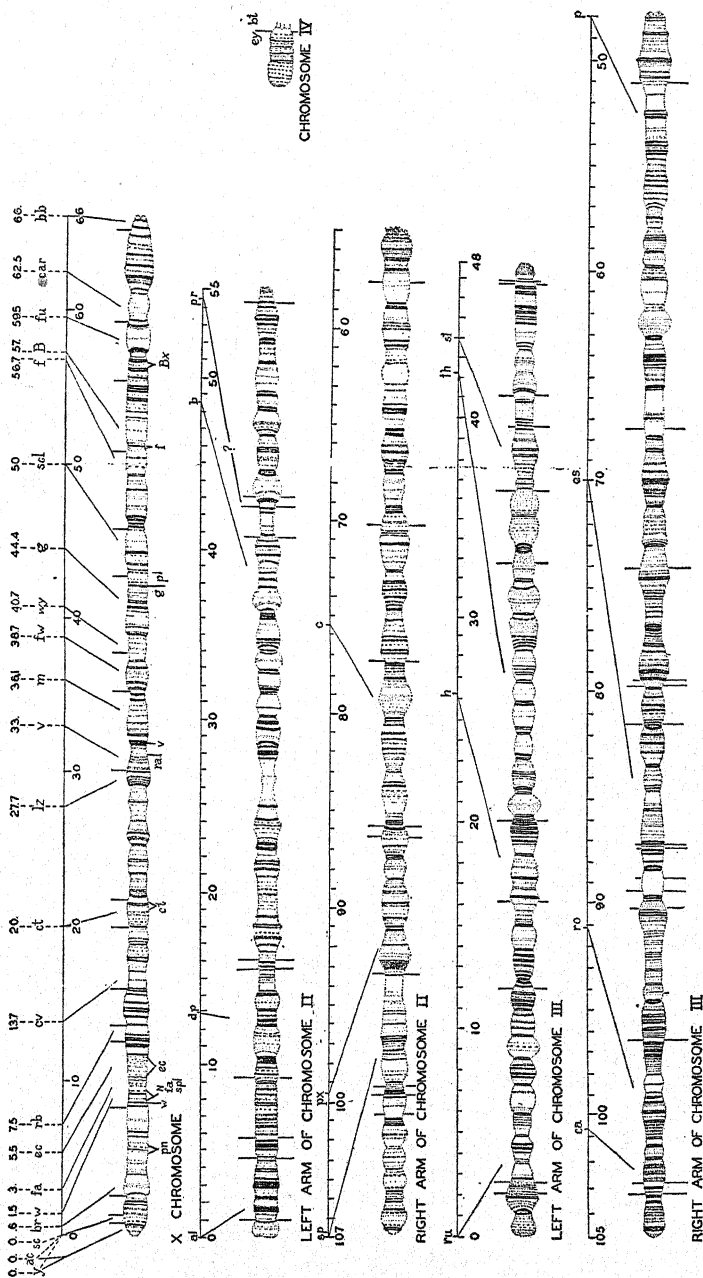
FIG. 117. Cytogenetic maps of the four chromosomes of Drosophila and the corresponding crossover maps. (From Painter in *The Journal of Heredity*.)

seen that the order of the genes is the same in the two maps. The distances between the genes correspond in general in the two maps, but the genes in the left end of the chromosome map are relatively crowded compared with those in the cytogenetic map. This crowding is probably due to the fact that crossing over is reduced in the left end of the X chromosome. Reduced crossing over would reduce the map distances and make it appear that the genes were closer together than they actually are. The part of the X to the right of *car* (carnation eyes) corresponds to the inert segment of the metaphase chromosome. It forms only a very small part of the total chromonema. But in the metaphase chromosome the inert segment is unwound and the rest of the chromonema is tightly wound up. Hence the inert segment forms a relatively large segment of the metaphase chromosome.

Cytogenetic maps have been made of the remaining chromosomes of Drosophila (the second, third, and fourth). They also confirm the correctness of the chromosome maps. It should be repeated here that the chromosome maps are constructed by means of crossover per cents, and they are based on the assumption that the per cent of crossing over is the same in any two segments of equal length. The cytogenetic maps show that this assumption on the whole is valid. Moreover, the cytogenetic maps confirm the chromosome theory of inheritance, for they show that the genes are located in definite parts of the chromosomes.

SUMMARY

1. It is possible to remove genes from different parts of a chromosome by X-ray treatment and then to determine what genes have been removed by means of linkage tests. The chromosomes can next be examined under the microscope in order to determine what segment has been removed. In this way we can determine what genes the segment contains. A drawing which shows the actual locations of the genes in a chromosome is called a *cytogenetic map*.

2. In Drosophila the chromosomes are very large in the salivary glands of the larva, and in addition they contain numerous cross bands which differ in a characteristic manner from one small segment to another, so that if a very small segment is removed, this can readily be seen under the microscope. The salivary gland chromosomes are therefore very favorable material for the construction of cytogenetic maps.

3. The chromosomes are permanently synapsed in the salivary gland

cells of a Drosophila larva. Moreover, each band in one chromosome pairs very closely with the corresponding band of a homologous chromosome.

4. A deleted segment, even if very small, can readily be detected in the salivary gland chromosomes of a deletion heterozygote, for the normal and the deleted chromosome lie side by side, and the normal chromosome is buckled opposite the deletion. The buckled segment corresponds to the one that is deleted.

5. In a deletion heterozygote of genotype $\dfrac{A \quad C}{a \ b \ c}$, the recessive gene b would express itself, and if the chromosome with the dominants had originally been ABC, we should know that the gene B had been deleted.

6. If genetic tests showed that a gene had been deleted from a chromosome, and if we then found that certain bands were missing from a salivary gland chromosome, it would follow that the gene was contained in the bands in question.

7. Inversions can be used for the construction of cytogenetic maps, as explained below.

8. In an inversion heterozygote, the normal and inverted chromosomes form a loop, and where the inverted segment enters and leaves the loop, its band sequence changes with reference to the normal. These changes in sequence mark the exact breakage points involved in the production of the inversion.

9. If a given gene, say, b, is outside an inversion $(ab \cdot cd \cdot ef)$, it undergoes normal crossing over with other genes on the same side of the inversion as it, in the inversion heterozygote. If it is inside the inversion $(a \cdot bcd \cdot ef)$, crossing over is suppressed between it and other genes in the inversion. Thus by linkage tests we can determine whether an inversion break is to the right of gene b $(a\,b \cdot c\ d \cdot e\,f)$ or to the left $(a \cdot b\ c\ d \cdot e\,f)$.

10. If linkage tests show that inversion I involves a break to the left of gene b $(a \cdot b\ c$, etc.$)$ and inversion II one to the right of b $(a\ b \cdot c$, etc.$)$, then gene b is included *between* the two breaks $(a \cdot b \cdot c$, etc.$)$. If microscopic examination now shows that certain bands are included between the two breaks in the salivary gland chromosomes of the inversion heterozygotes, it follows that gene b is represented by one or more of these bands.

11. Translocations can be used for the construction of cytogenetic maps as described below.

12. In a translocation heterozygote the two normal and the two translocated chromosomes form a four-armed figure. A given translocated chromosome has one segment in one arm and the other in another arm, and where it leaves one arm and enters the other, the band sequence changes with reference to the normal. This change in sequence marks the exact breakage point in the translocated chromosome in question.

13. By means of linkage tests it can be determined whether a given gene (say, b) is to the right of the breakage point in a translocated chromo-

some $(a \cdot b \ c \ d)$ or to the left $(a \ b \cdot c \ d)$. Hence if it is to the right of the breakage point in one translocation and to the left in another, then it is located between the two breakage points $(a \cdot b \cdot c \ d)$. If microscopic examination now shows that certain bands are included between the two breaks, in the salivary gland chromosomes of the translocation heterozygotes, it follows that gene b is represented by one or more of these bands.

14. The principle involved in correlating genes with the bands of the salivary chromosomes is the same whether deletions, inversions, or translocations are used in making the correlation. In all cases, when genetic tests show that certain genes are removed from their normal position, microscopic examination shows that certain bands are also removed. Hence the genes are represented by the bands.

15. Chromosome maps (as contrasted to cytogenetic) are constructed by means of crossover per cents, and they are based on the assumption that in any two segments of a chromosome of equal length, the per cent of crossing over is the same. This assumption on the whole is valid, for when the chromosome maps and the cytogenetic maps are compared, it is found that on the whole the spacing of the genes agrees in the two.

PROBLEMS

1. In the "metaphase" X chromosome of Drosophila the active section of the chromonema is tightly wound up but the inert section is not. What effect does this have on the apparent size of the inert region, compared to the active region, in the metaphase chromosome?

2. Suppose that ordinarily there was 1 per cent of crossing over between two genes one physical unit apart (say, one ten-millionth of an inch apart). Suppose further that two genes were 10 physical units apart but there was reduced crossing over in the section of the chromosome in which they were located and that there was only five per cent of crossing over between them. Then how would the relative distance apart of these genes compare on the chromosome map and the cytogenetic map?

3. In a map of the X chromosome of Drosophila the genes are relatively crowded at the left hand end of the map. Assuming that actually the genes are about evenly distributed over the active portion of the X, how might we account for this crowding of the genes at the left end of the chromosome map?

4. Given two translocations, A and B, between the third and fourth chromosomes of Drosophila, both involving a transfer of a left segment of the third chromosome to the fourth. In A bands 61–71 in the salivary gland chromosome are transferred to the fourth, and in B bands 61–74 are transferred. Linkage tests show that in A a certain marker gene (call it m) is not transferred to the fourth chromosome; in B, it is. Give the location of m in the salivary gland chromosome.

15. *GENIC BALANCE AND CHROMOSOME NUMBER*

$\mathbf{I}$N DISCUSSING sex determination we saw that the normal development of sex was dependent upon a normal genic balance; that is to say, the X and the autosomes had to be together in definite proportions to one another. A departure from these proportions causes abnormal sex development, as seen in intersexes.

All other traits besides sex are dependent upon a certain genic balance for their normal development, insofar as the chromosomes must be present in a definite proportion to one another. Ordinarily there are two chromosomes of each kind in an unreduced cell, so that the normal proportions' are two of a given kind to two of each of the remaining kinds. But if, for example, there should be three of a given kind to two of the rest, the amount of the kind in question would be greater, relative to the rest, than normal. This relative excess would represent genic unbalance. It might express itself by causing abnormal development, and the animal or plant which showed the resulting abnormal traits would be regarded as a mutant.

A mere increase in chromosome number would, however, not necessarily result in genic unbalance. Thus if a cell normally had two chromosomes of each kind, then four of each kind would represent no change in their relative proportions. Accordingly, there would be no disturbance in the genic balance, and the plant or animal with the doubled number would appear normal, at least for the most part. It would, however, be regarded as a mutant because it does not have the normal chromosome number.

Tetraploids.—The number of chromosomes in a cell is sometimes accidentally doubled through irregular cell division. Thus in Fig. 118 we begin with a cell which contains two sets of chromo-

somes (or 2*n*), each set consisting of three chromosomes (a long, a medium, and a short). The chromosomes then split into two, as they do when cell division is about to take place. But suppose now that cell division failed to follow upon chromosome division. Then the cell would permanently have four sets of chromosomes, and it would be referred to as *tetraploid* (or 4*n*), in contrast to

A. DIPLOID (2*n*) B. CHROMOSOME DIVISION (NOT FOLLOWED BY CELL DIVISION) C. TETRAPLOID (4*n*)
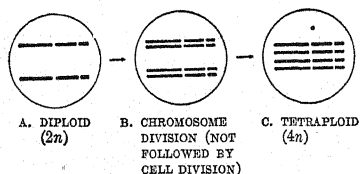
Fig. 118. The origin of a tetraploid.

the normal diploid (or 2*n*). In brief, then, a tetraploid cell might arise as the result of chromosome division not followed by cell division.

A tetraploid cell might after a period of rest start to divide again. In this event the chromosomes would first split into two and line up in the middle of the cell, but four of each kind would split and line up instead of two. Cell division might now proceed to completion. The split halves of each chromosome would then pass to opposite poles and two cells would be formed, each with four chromosomes of each kind. Further cell division might result in a larger number of cells of similar character. Once the chromosome number has accidentally got doubled, it remains double from one cell division to the next.

Assume now that the chromosome number had got doubled in a cell of a growing plant and that this cell by further division gave rise to a flower. Then all the unreduced cells of the flower would be tetraploid. Before the flower formed its gametes, the reduction division would take place as usual; but four chromosomes of each kind would come together in the middle of the cell. Hence two of each kind would go to one pole and two to the other. Thus the gametes would come to contain a double set of chromosomes (the diploid number) instead of a single set (the haploid number). If the flower under discussion were now self-fertilized, two diploid gametes would combine and form a fertilized egg. This would contain four chromosomes of each kind (two coming from each gamete), and it would develop into a tetraploid plant. From this a tetraploid race might arise.

Tetraploids are seldom found in the animal kingdom, but they are not at all uncommon in the plant kingdom. There are tetraploid races of the plum, the strawberry, the tomato, the Jimson weed (Datura), and many other plants. Figure 119 shows a tetraploid

tomato and a normal diploid for comparison (and in addition a "triploid" to be considered later). In most respects the tetraploid is normal. This is as expected on the theory of genic balance. But tetraploids often have somewhat larger cells than normal diploids, and sometimes the plant as a whole and its parts are somewhat larger than normal. Occasionally they are somewhat more vigorous.

In a tetraploid there is somewhat of a tendency for the reduction divisions to be irregular, because sometimes three chromosomes
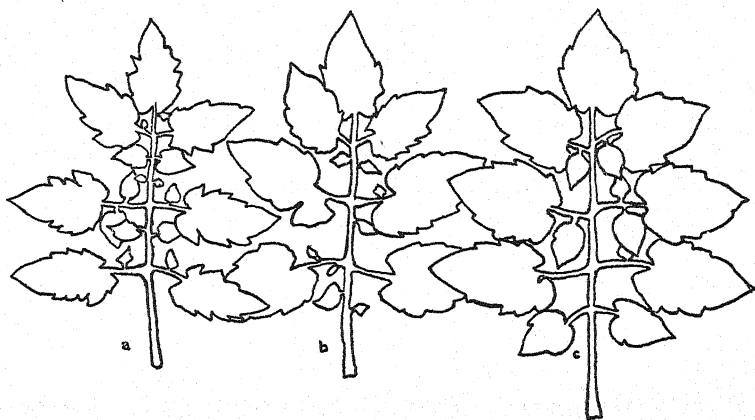
Fig. 119. Typical leaves of diploid (a), triploid (b), and tetraploid (c) tomato plants. (From Jorgensen in *The Journal of Genetics*.)

of a kind go to one pole and only one to the other. As a result a pollen cell occasionally contains one or more kinds of chromosomes in triploid or haploid amount and the rest in diploid amount. Such a cell is genically unbalanced. Pollen cells are very susceptible to genic unbalance (more so than eggs) and they die early after their formation or are incapable of growth down the pistil. The fertility of a tetraploid plant might therefore be somewhat reduced.

Tetraploids sometimes arise spontaneously, but they might also be produced artificially by a variety of agents. Belling and Blakeslee have shown that tetraploids can be produced by treating the buds of Datura with a weak solution of the drug *colchicine*. The buds are sprayed with the solution or are dipped in it, or the seeds are soaked in it for about a day. Colchicine is now being exten-

sively used for the production of tetraploids in other species besides Datura. Horticulturists are using it to produce large-flowered races of garden plants. Cold and other "shock" treatment might also produce tetraploids. Whatever might be the kind of treatment employed for the production of tetraploid races, the way in which it produces them is usually the same. A tetraploid cell is first formed in one or a few cells of a bud or a seed. This happens as the result of chromosome division not followed by cell division. From the single tetraploid cell one or more flowers are derived through cell division. The flowers have diploid gametes, and by their union the gametes give rise to tetraploid plants.

The Relative Absence of Tetraploid Races in Species with Separate Sexes.—In species in which the sexes are separate, newly arisen tetraploid tissue might give rise to sperm cells or to egg cells but it could not give rise to both within the same individual since the sexes are separate. Therefore the diploid gametes formed from the tetraploid tissue would have to combine with the normal haploid gametes of an individual of opposite sex, and this would produce offspring with three sets of chromosomes instead of four. In animals the sexes are usually separate and for this reason tetraploid races usually find it difficult or impossible to get a start in animals.

But even if tetraploids should happen to get a start in the animal kingdom, they would as a rule be sterile. Suppose for example that we were dealing with an animal in which the female normally has two X's and the male an XY pair, as in Drosophila and man. Then a tetraploid female would have four X's and a tetraploid male two X's and two Y's. At the reduction division in the female two X's would pair with two other X's, with the result that two would go to one pole and two to the other. Hence all the eggs would contain two X's. But in the male an X would pair with an X as a rule, and a Y with a Y. Hence the sperm cells would receive an X and a Y. The combination of an XX egg cell and an XY sperm cell would give rise to $XXXY$ offspring. These would have four autosomes of each kind (since the parents were tetraploid), and to be normal in sex they should have either 2 X's or 4 X's. But instead they would have 3 X's. Hence they would be intersexes and would be sterile, and the race would disappear through sterility. This would apply rather generally to tetraploid races in any species with separate sexes.

In summary, then, tetraploids are usually absent in animals or plants with separate sexes because (1) tetraploids could seldom arise when the sexes are separate and (2) if tetraploids did happen to arise, they would produce offspring of composition $XXXY$ and these would as a rule be sterile intersexes. As the sexes are usually separate in animals we could thus account for the rarity of tetraploid races in the animal kingdom. But the sexes are combined in some animals and in them we sometimes find tetraploid races.

A notable exception to the above statements has been found by Warmke in Melandrium, a flowering plant with separate sexes. In Melandrium the Y plays an important role in sex determination in that it reacts with the X to produce a male, and one Y in combination with 3 X's produces a male as a rule. An $XXXY$ male produces two classes of sperm cells: XX and XY. These on fertilizing the XX eggs produce $XXXX$ and $XXXY$ offspring, or females and males, both fertile. Thus Melandrium can give rise to a fertile tetraploid race, even though the sexes are separate. But in most organisms, particularly animals, sex is determined not by the interaction of the X and the Y but by the ratio of X to autosomes, and in them the $XXXY$ offspring would as a rule be sterile intersexes.

Mendelian Ratios in Tetraploid Races.—In Datura white flower color (w) is recessive to the normal purple ($+$). A tetraploid contains four genes at each locus because it has four chromosomes of every kind. A white tetraploid Datura would therefore be $\dfrac{w}{w}\Big|\dfrac{w}{w}$ and a pure purple $\dfrac{+}{+}\Big|\dfrac{+}{+}$. If the two were crossed, the F_1 would be $\dfrac{w}{+}\Big|\dfrac{w}{+}$. In Fig. 120 the chromosomes of the F_1 are lined up and numbered (1)–(4). When the reduction division takes place, a given chromosome, say, (1), might go to one pole together with any one of the remaining three, so that the pole in question might receive three possible combinations; namely, (1) (2), (1) (3), or (1) (4). In Fig. 120 these combinations are shown at the left pole of each division. The other pole would in each instance receive the remaining two chromosomes. The two poles together contain every possible combination of two chromosomes at a time; namely, (1) (2), (1) (3), (1) (4), (2) (3), (2) (4), (3) (4). Accordingly, the hybrid forms its gametes in the ratio of $1 + + : 4 + w : 1\,w\,w$, as can be seen by adding up the gametes of like classes in Fig. 120.

Thus out of every six gametes, only one would contain only w. Therefore, when the hybrid was self-fertilized only one combination in 36 would involve two gametes both of class ww and give rise to an offspring of class $\dfrac{w}{w}\Big|\dfrac{w}{w}$. This class would be white. The rest of the offspring (35 out of every 36) would receive one or more $+$'s, and as one $+$ is sufficient to make a plant purple, the rest would all be purple. Thus the F_2 offspring would tend to be produced in the ratio of 35 purple : 1 white. This is to be contrasted to the 3 : 1 ratio in the F_2 when two diploids are crossed.
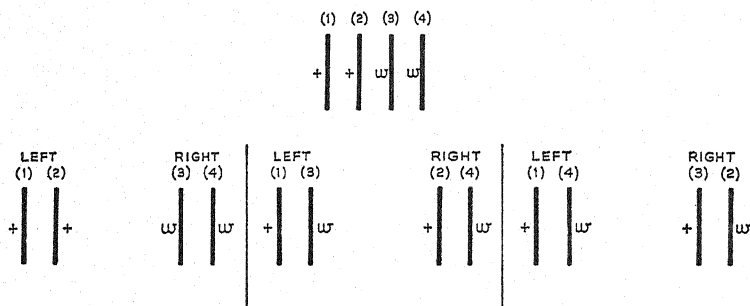
Fig. 120. The reduction division in a tetraploid of genotype $\dfrac{w}{+}\Big|\dfrac{w}{+}$.

A tetraploid Datura hybrid for white might be crossed to a pure white, thus $\dfrac{w}{+}\Big|\dfrac{w}{+} \times \dfrac{w}{w}\Big|\dfrac{w}{w}$. This would be the test cross. The hybrid would as before form three classes of gametes in the ratio of $1\ ++ : 4\ +w : 1\ w\,w$. The white plant would form just one class of gametes; namely, ww. At fertilization these would combine with the gametes of the hybrid $(1\ ++ : 4\ +w : 1\ ww)$, giving offspring in the ratio of $1\ \dfrac{+}{w}\Big|\dfrac{+}{w} : 4\ \dfrac{+}{w}\Big|\dfrac{w}{w} : 1\ \dfrac{w}{w}\Big|\dfrac{w}{w}$, or 5 purple : 1 white. This is to be contrasted to the 1 : 1 ratio into which the offspring fall when a diploid hybrid is crossed to a white.

The above ratios (35 : 1 and 5 : 1) are not exactly realized in the F_2 when tetraploids are crossed, because the reduction divisions are somewhat irregular in a tetraploid, and accordingly the hybrid does not form its gametes in exactly the ratio given.

Allo-tetraploids.—Figure 121, top left, shows the union of two gametes, one from species *A*, the other from a somewhat closely related species *B* (assuming that the two species can be crossed).
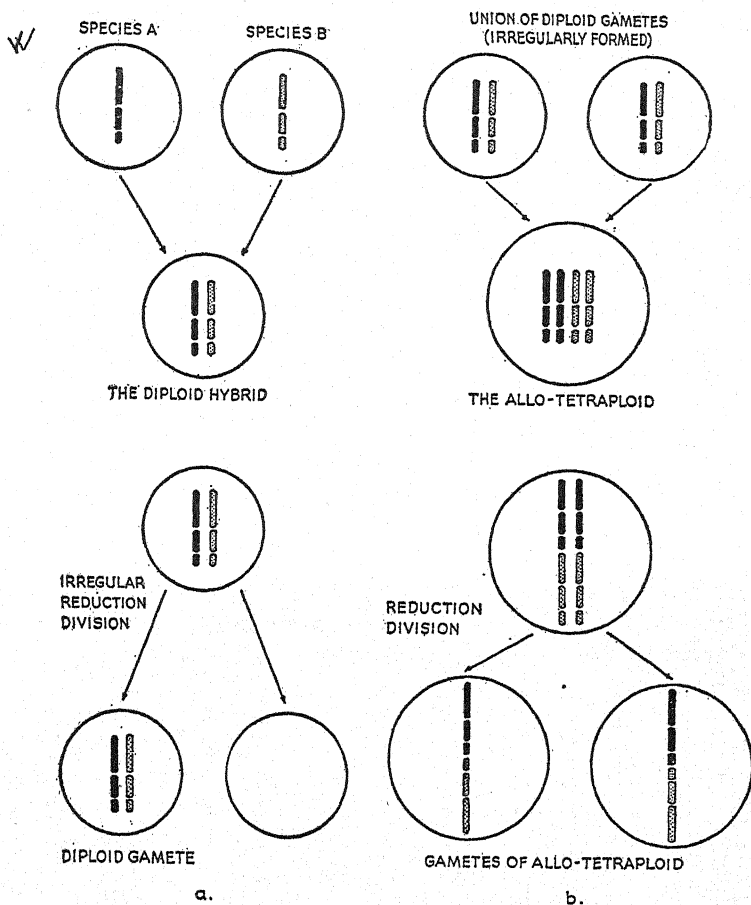
FIG. 121. The origin of an allo-tetraploid.

Each gamete is shown with a set of three chromosomes (long, medium, and short). Since the two species are somewhat closely related, their chromosomes are alike in many respects but also different (as indicated by the difference in the shading). The hybrid formed by the cross has two sets of chromosomes (one set from

each parent species)—it is diploid. Assume now that the chromosomes in the two sets are not sufficiently alike to attract one another at the reduction division. Then they will not pass into the gametes in an orderly manner in the hybrid. As a rare accident, both sets might enter one gamete (Fig. 121a, bottom). If two such gametes happened to combine, they would form a hybrid with four sets of chromosomes, two from species A and two from species B (Fig. 121b, top). Since this hybrid would have four sets of chromosomes, it would be a tetraploid, but it would be designated as an *allo-tetraploid* in order to indicate that two sets came from one species and two from another (*allo* denoting other). Ordinarily when a race contains four sets of chromosomes, all four belong to the same species (as in a Datura tetraploid), and such a race is sometimes referred to as an *auto*-tetraploid (*auto* denoting self or same).

Figure 121b, bottom, shows the reduction division in the allo-tetraploid under discussion. Set A pairs with set A, and B with B. Therefore every gamete contains one complete A set and one complete B set. When two such gametes combine they form offspring with two A sets and two B sets, or allo-tetraploids. Hence the allo-tetraploid is fully fertile and breeds true.

Karpechenko produced an allo-tetraploid by crossing the radish (*Raphanus sativus*) and the cabbage (*Brassica oleracea*). The hybrid formed by crossing these two species is itself a diploid—it contains only one set of radish chromosomes (R) and one set of cabbage chromosomes (C) and it can be designated as RC. It is almost completely sterile, because radish and cabbage chromosomes are so different that they fail to attract one another at the reduction division. But the hybrid forms an occasional gamete which contains one complete set of radish chromosomes and one complete set of cabbage chromosomes. When two such gametes combine they produce a plant which contains two sets of radish chromosomes and two sets of cabbage chromosomes ($RRCC$). This is an allo-tetraploid. It combines traits of both the radish (Raphanus) and the cabbage (Brassica) and has been named *Raphano-brassica* (Fig. 122.1).

There are nine chromosomes to a set in both the radish and the cabbage. In *Raphano-brassica* ($RRCC$) there are four sets of nine, or 36 chromosomes in all. At the reduction division, the nine chromosomes in one radish set pair with the nine radish in

the other set, and likewise for the two cabbage sets. Therefore every gamete receives one complete set of radish chromosomes and one complete set of cabbage (or *RC*). When two such gametes combine, they produce offspring of composition *RRCC*. Thus *Raphano-brassica* is fully fertile and breeds true. This is in contrast to the genetic behavior of the diploid hybrid (*RC*), which we saw was highly infertile.

It is possible to produce an allo-tetraploid artificially with the aid of colchicine. Thus, suppose we had first produced a diploid hybrid, as shown in Fig. 121, top left. We might then treat the hybrid with colchicine, say, while it was still a seed, and so we might cause a doubling of the chromosome number in one of the cells of the seed. This cell would have four sets of chromosomes (two of species *A* and two of species *B*). It might give rise to an allo-tetraploid flower (by cell
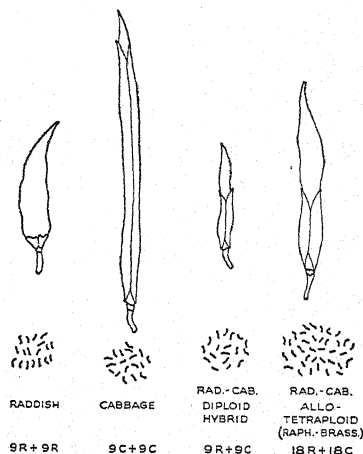
FIG. 122.1. The origin of *Raphano-brassica*. (From Karpechenko in *Zeit. f. ind. Abst. u. Ver.*)

RADDISH — CABBAGE — RAD.-CAB. DIPLOID HYBRID — RAD.-CAB. ALLO-TETRAPLOID (RAPH.-BRASS.)

9R+9R — 9C+9C — 9R+9C — 18R+18C

division), and the flower upon being self-fertilized would produce allo-tetraploid offspring. In the present instance the original chromosome doubling occurred in a vegetative cell; in the previous case (the radish-cabbage cross) it occurred in the gametes. Attempts are now being made to get allo-tetraploids from hybrids between various species of plants, as wheat and rye, by means of colchicine treatment. Often the diploid hybrids themselves are very infertile. But the allo-tetraploids should sometimes be fertile and some of them might prove to be of great economic value.

Polyploids.—There might be a doubling of the chromosome number in a bud of a tetraploid plant. Since the tetraploid has four sets of chromosomes (or $4n$), the doubling would result in a cell with eight sets of chromosomes (or $8n$), and so an $8n$ race might arise. A further doubling might produce a $16n$ race, but 16 is about the limit to the number of sets possible in a cell.

A diploid plant might cross with a tetraploid. In this event a

$1n$ gamete (from the diploid) would combine with a $2n$ gamete (from the tetraploid), and the offspring would be $3n$. A $6n$ race might now arise as the result of a doubling in the $3n$ plant; or it might arise by the combination of a $2n$ gamete (from a $4n$ race) and a $4n$ gamete (from an $8n$ race). It is evident from this that plants having various numbers of sets might come into existence. A plant which has more than two sets of chromosomes is called a *polyploid*.

It is sometimes found that the chromosome number differs within a related group of plants. Thus some kinds of wheat have fourteen chromosomes but other kinds have twenty-eight or forty-two. All these numbers are multiples of seven. In the kind of wheat with fourteen chromosomes there are two sets of seven. This is the ordinary diploid race. The kind with twenty-eight chromosomes has four sets of chromosomes ($4n$). This was probably derived from the one with two sets ($2n$) by a doubling of the chromosome number. Ordinary wheat (Triticum vulgare) is $6n$ and has 42 chromosomes (6×7). It is possible to produce a $6n$ race of wheat artificially by first getting a $3n$ plant and then doubling the chromosome number by means of colchicine.
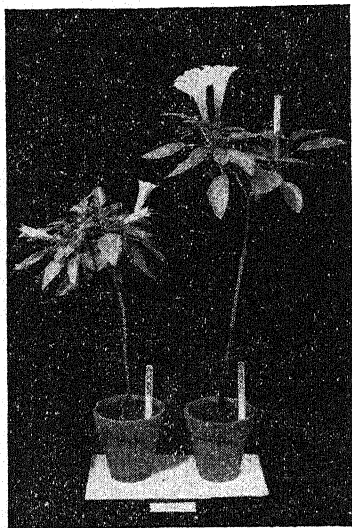
FIG. 122.2. A haploid Datura (left) with a normal diploid for comparison. (From Satina, Blakeslee, and Avery in *The Journal of Heredity*.)

According to Darlington, over half the species in the plant kingdom are polyploids. This would indicate that polyploids can arise with relative ease in the case of plants. In the animal kingdom, however, polyploids are comparatively rare, for the reasons given in connection with tetraploids.

Haploids.—In Datura an egg of a diploid plant occasionally develops without fertilization. As the egg itself is haploid, it gives rise to a haploid plant; that is, a plant that has only one set of chromosomes in each of its vegetative cells (Fig. 122.2). Haploid

plants are fairly normal in appearance because each chromosome exists in normal proportion to the rest. However, the reduction divisions are very irregular in a haploid as a result of the fact that the cell has just one set of chromosomes. Normal reduction requires the pairing of chromosomes, and when the chromosomes exist singly they cannot pair. Instead they line up singly in the middle of the cell and as a rule part of the set passes to one pole, part to the other. Hence few or no gametes are formed with a complete set of chromosomes, and the plant is sterile.

Triploids.—Figure 123a shows the union of two gametes, one containing one set of three chromosomes, the other two sets. The
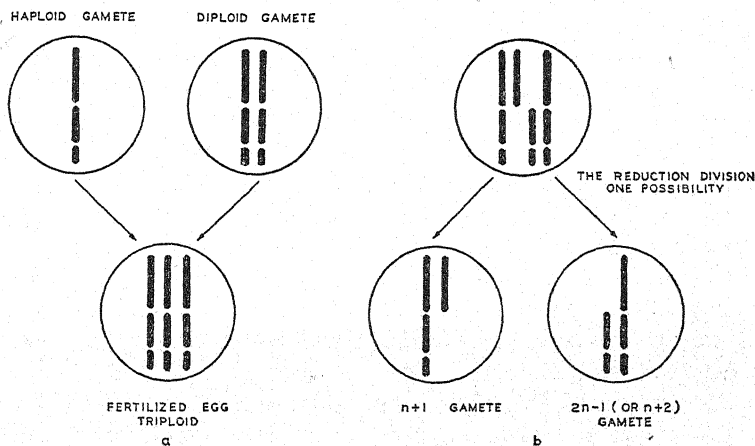
FIG. 123. The triploid.

gamete with one set would normally be derived from a diploid parent, and the one with two sets from a tetraploid. The offspring contains three sets ($3n$) and is called a *triploid*.

When the reduction division takes place in a triploid, three chromosomes of each kind are potential partners and as a rule one of the three goes to one gamete and two go to the other (Fig. 123b). But one of every kind does not necessarily go to one and the same gamete and two to the other. Thus in Fig. 123b two of the long kind go to the left gamete but only one of each of the other two kinds. This particular gamete would therefore contain one complete set and in addition one extra chromosome (a long one), and it would be referred to as an $n + 1$ gamete. The right-

hand gamete in Fig. 123b might be described either as $2n - 1$ (two sets minus one) or $1n + 2$ (one set plus two). Figure 123b shows only one possible kind of reduction division in the triploid under consideration. The left-hand gamete might have received either two medium or two short chromosomes and one of each of the rest. Obviously there are as many possible kinds of $n + 1$ gametes as kinds of chromosomes (3 in the present instance). Moreover, if the diploid contained, say, 12 kinds of chromosomes, then a given gamete might receive one set of chromosomes plus any number of extras up to 12 ($n + 1$, $n + 2$, $n + 3$, etc.). Very few gametes would receive just $1n$ (with no extras) or $2n$ (two complete sets). It is obvious therefore that the triploid would produce very few triploid offspring when self-fertilized. It might, however, reproduce asexually, and in nature there are triploids which maintain themselves asexually.

Trisomics.—Figure 124 shows the union of an $n + 1$ gamete and a $1n$ gamete. The offspring is $2n + 1$. It contains three chromosomes of one kind (long in Fig. 124) and accordingly is called a *trisomic*. Either one of the other two chromosomes instead of the long might have been present in extra amount so that there are as many possible kinds of trisomics as kinds of chromosomes (three in the present instance). A trisomic, then, is a plant which contains three chromosomes of a given kind but only two of each of the rest.

We just saw that a triploid produces $n + 1$ gametes, and if the triploid were crossed a normal diploid, the $n + 1$ gametes of the triploid would combine with the n gametes of the diploid and produce $2n + 1$ offspring. Thus it is readily possible to get trisomics by crossing a triploid and a diploid. But trisomics might also be produced accidentally by normal diploids. For when the reduction division takes place in a diploid, it sometimes happens that both members of a pair of chromosomes pass to one pole and neither to the other (Fig. 125). This is known as *non-disjunction*. If only one chromosome pair undergoes non-disjunction, then the two resultant gametes are $n + 1$ and $n - 1$ respectively. The $n + 1$ gamete and a normal (n) gamete would, by combining, give rise to a $2n + 1$ offspring (a trisomic). But non-disjunction is comparatively rare in normal diploids, and it is much easier to get trisomics from triploids than directly from diploids.

In Datura trisomics can readily be made to order by first crossing

a diploid and a tetraploid and so producing a triploid. This then crossed to a normal diploid produces trisomics of all possible kinds. However, trisomics do occasionally arise in Datura as a result of non-disjunction.

It is possible to get $2n + 1$ types in which the extra chromosome contains an abnormal arrangement of segments as the result of a translocation. A trisomic, however, is a $2n + 1$ type in which one
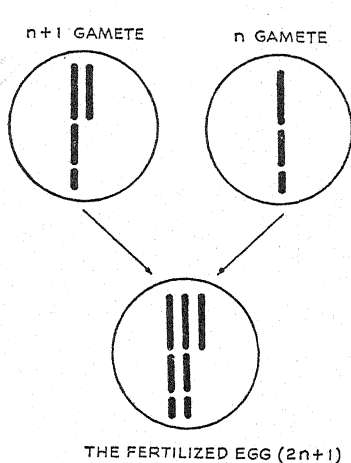
FIG. 124. The origin of a trisomic $(2n + 1)$.
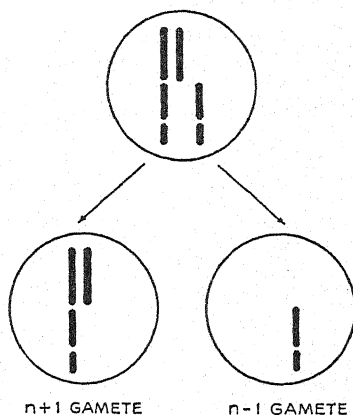
FIG. 125. Non-disjunction.

of the normal, or *primary*, chromosomes is present in extra amount, and it is therefore known as a *primary* $2n + 1$ type. In Datura there are twelve kinds of chromosomes, and therefore twelve possible kinds of trisomics or primary $2n + 1$ types. All of these are genically unbalanced.

The Effects of Genic Unbalance in Datura.—The effects of genic unbalance have been studied by Blakeslee and Belling in Datura. Some of these effects are shown in Fig. 126. A normal seed capsule is shown in the upper part of the figure, and each of the twelve possible trisomics below. The capsule labeled 1·2, for example, comes from a trisomic plant which has three doses of a chromosome labeled 1·2 but two of the rest. The abnormal capsule is in a sense a mutant trait, but it is due entirely to a disturbance in genic balance, not to any mutant genes. Many other parts of the plant besides the capsule are abnormal. Each trisomic
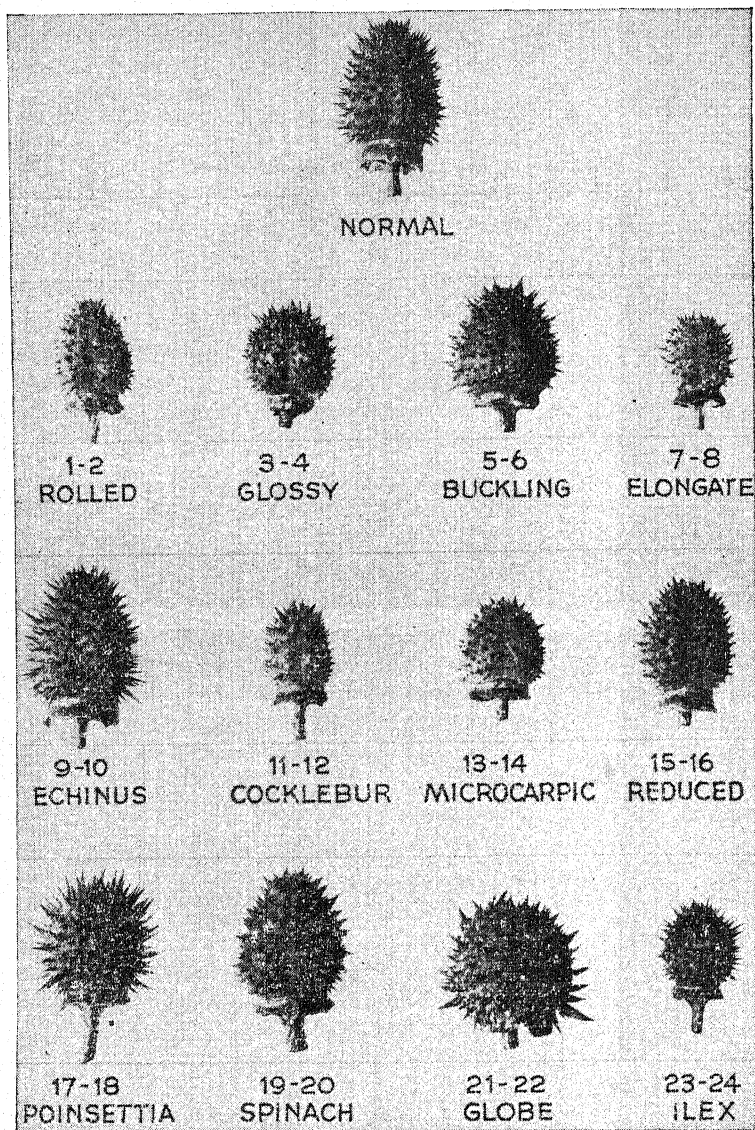
FIG. 126. Trisomics in Datura, illustrating the effects of genic unbalance
(From A. F. Blakeslee in *The Journal of Heredity*.)

has a distinctive set of mutant traits and has received a name (rolled, glossy, buckling, etc.).

In a $2n + 1$ plant approximately half the gametes are $n + 1$, and if two such gametes combined they would produce a $2n + 2$ offspring; that is, a $2n$ which contains two extra chromosomes of a given kind, making four of the kind in question (a *tetrasomic*). This would obviously be more unbalanced than the corresponding $2n + 1$. Figure 127 shows the capsule of a normal plant ($2n$) and the capsules of a $2n + 1$ and a $2n + 2$ plant, the last two having the same kind of chromosome in extra amount ($21 \cdot 22$).

NORMAL($2n$) $2n + 1$ $2n + 2$
(ONE EXTRA 21·22) (TWO EXTRA 21·22'S)
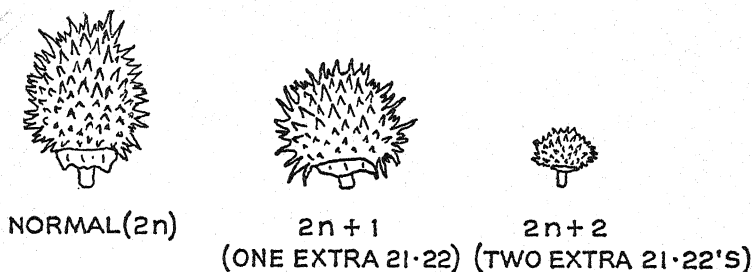
Fig. 127. Different degrees of genic unbalance in Datura. (From Blakeslee in *The Journal of Heredity*.)

Secondary and Tertiary $2n + 1$ Types.—In Datura a translocation sometimes involves two chromosomes of the same kind. For example, two $1 \cdot 2$'s might each break into segments 1 and 2. The two 1's might then come together and so might the two 2's, giving $1 \cdot 1$ and $2 \cdot 2$. A plant which is otherwise a normal diploid might now come to contain an extra chromosome of composition $1 \cdot 1$ or $2 \cdot 2$. It would in other words be a $2n + 1$ type in which the extra chromosome was made up of one half doubled. Such a plant is called a *secondary* $2n + 1$ type in contrast to a primary. For each primary there are two possible secondaries. Figure 128 shows a primary type ($2n + 1 \cdot 2$) and the two corresponding secondaries ($2n + 1 \cdot 1$ and $2n + 2 \cdot 2$). The secondary on the left ($2n + 1 \cdot 1$) has two extra doses of segment 1 as compared to one extra dose in the primary, and it is therefore more unbalanced for segment 1 than the primary. In like manner, the secondary on the right ($2n + 2 \cdot 2$) is more unbalanced for segment 2 than is the primary. The primary type ($2n + 1 \cdot 2$) is intermediate between the secondaries, because
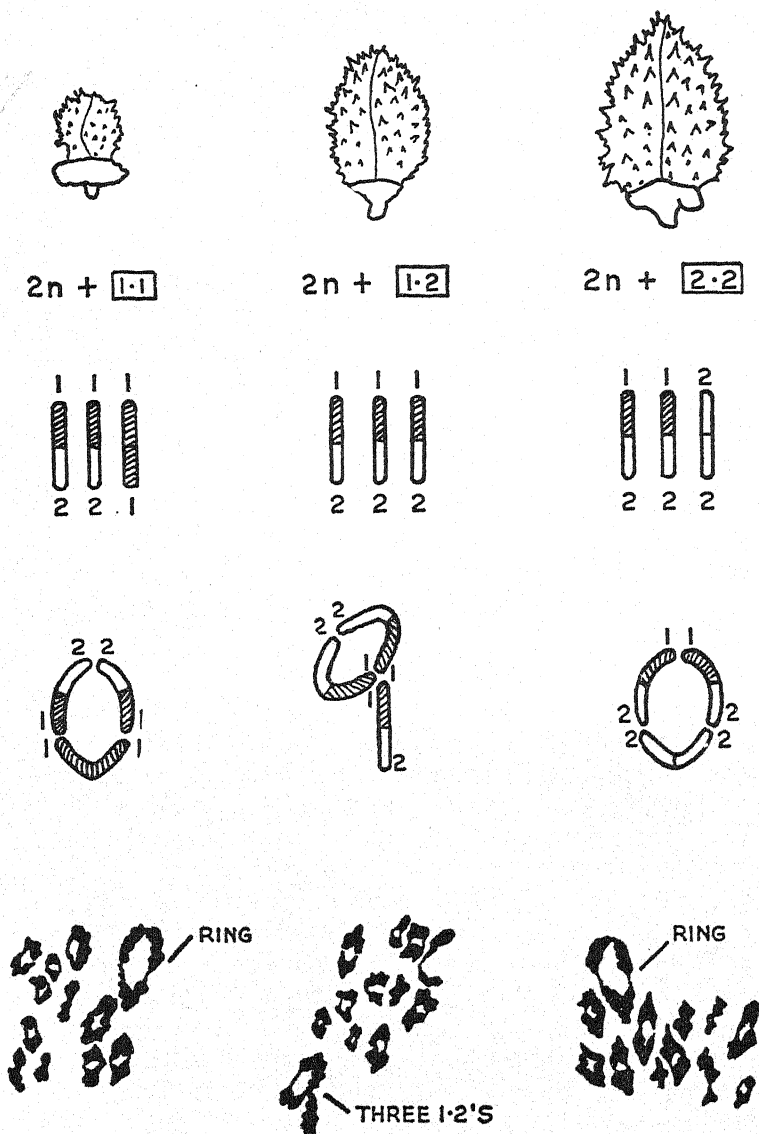
FIG. 128. A primary and its two secondaries in Datura. (From Blakeslee in *The Journal of Heredity*.)

it shows the combined effects of both segments (1 tending to make the capsule small, 2 large, and 1·2 intermediate).

Figure 128, third row, shows how two 1·2's and a 1·1 come together at the reduction division. The ends of like segments are paired and the three chromosomes form a ring (shown in bottom row as they appear in an actual specimen). Two 1·2's and a 2·2 would also form a ring. Hence in each of the two secondaries derived from chromosome 1·2 there is a ring of three chromosomes. In the primary type there are three 1·2's and all three are potential partners at both ends, but often one dangles from one end.

Sometimes translocations involve two different kinds of chromosomes in Datura, as 1·2 and 17·18. Accordingly, we might get two new chromosomes 1·18 and 2·17. It would then be possible to get a $2n + 1$ plant in which either 1·18 or 2·17 was the extra chromosome. Such plants are called *tertiary* $2n + 1$ types. A $2n + 1·18$ plant would have 3 doses of segment 1 (two in the pair of 1·2's and one in 1·18) and three doses of segment 18 (2 in the pair of 17·18's and one in 1·18), but it would have only two doses of all other segments. It would therefore have certain mutant traits, due to the relative excess of segments 1 and 18. The mutant traits in question would be those found partly in a $2n + 1·2$ plant and partly those found in a $2n + 17·18$ plant.

Mendelian Ratios from Crosses Involving Trisomics.—In Datura the gene for white flower (w) is in the chromosome numbered 17·18, and this particular chromosome is represented three times in the trisomic called "Poinsettia" (Fig. 126). The normal flower color is purple and therefore a pure purple Poinsettia contains three doses of the normal allele of white, or $+\left|\overset{\displaystyle +}{+}\right.$. Approximately half the gametes contain two 17·18's and the other half one 17·18, and they are accordingly $+ +$ and $+$, respectively. If a purple Poinsettia should be crossed to a diploid white ($+\left|\overset{\displaystyle +}{+}\right. \times w/w$), then a $+ +$ gamete might combine with a $\overset{\displaystyle w}{w}$ gamete. Thus half the F_1 offspring would be $+\left|\overset{\displaystyle +}{+}\right.$. These would be Poinsettia (since they have three 17·18's), but they would be hybrid purples. When the reduction division takes place in the hybrid, three 17·18's are potential partners, as shown in the upper part of Fig. 129, in which the chromosomes are numbered

(1)–(3). Therefore, when the chromosomes separate (1) might go to one pole and (2) and (3) to the other (lower left part of figure); likewise (2) might go to one pole or (3) might, and the remaining two in each instance to the other pole. The resulting gametic ratio is $1 + + : 2 + w : 2 + : 1\,w$. However, those classes with two chromosomes $(1 + +$ and $2 + w)$ are not formed quite as often as expected because at the reduction division one of the
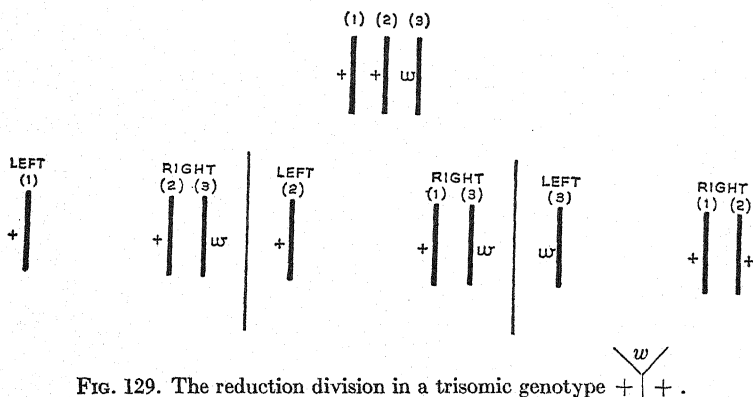
FIG. 129. The reduction division in a trisomic genotype $+ \mid + $.

two chromosomes sometimes gets lost by lagging in the middle of the dividing cell and remaining in between the products of division.

If the hybrid Poinsettia were crossed to an ordinary diploid white (w/w), then one w would be added to each of the above classes of gametes $(1 + + : 2 + w : 2 + : 1\,w)$, and the offspring would tend to approximate the ratio of $1 + + w : 2 + w\,w : 2 + w : 1\,w\,w$. As one $+$ is enough to make a plant purple, the first three classes would appear purple and the last white, giving an approximate ratio of 5 purple : 1 white. This is to be contrasted to the 1 : 1 ratio when an ordinary diploid hybrid is crossed to a white plant $(w/+ \times w/w)$.

If the hybrid Poinsettia were self-fertilized, it would produce offspring in the ratio of 35 purples : 1 white. This follows from the fact that in the hybrid only one gamete in six is w (since the gametic ratio is $1 + + : 2 + w : 2 + : 1\,w$), and hence only one combination in 36 produces plants pure for white (w/w). The whites would all be w/w and would have only two doses of chromosome $17 \cdot 18$, and they would therefore be normal diploids (not

Poinsettia). Among the purples some would have two doses of 17·18 (normal diploids), others three doses (Poinsettias), still others four doses (extreme Poinsettias). We can refer to 5 : 1 and 35 : 1 as *trisomic ratios*.

The Genetic Identification of Datura Chromosomes.—We do not know in advance of the experimental evidence that the gene white (w) is in chromosome 17·18 in Datura. But when we cross a purple Poinsettia to a diploid white, we get a trisomic ratio in the F_2 as just described, and so we know that white (or its normal allele $+$) is in the chromosome that is represented three times in Poinsettia. This chromosome has arbitrarily been numbered 17·18. All trisomics other than Poinsettia would have 17·18 in only diploid amount, and if they were hybrid for white, they would produce offspring in the usual Mendelian ratio (1 : 1 on being test crossed, or 3 : 1 on being selfed).

In order to determine therefore in what chromosome of Datura a given mutant gene is, we should cross a diploid plant pure for that gene to each of the twelve trisomic mutants, and then test cross (or self-fertilize) each of the twelve F_1 trisomics. We should then find that some one of the twelve F_1 produced offspring in the trisomic ratio, and we should conclude that the mutant gene was in the chromosome which the trisomic plant in question had in triple amount. The twelve chromosomes of Datura are somewhat distinct in size and shape, and so it is possible to identify them under the microscope. We can therefore tell which particular chromosome a given trisomic carries in triploid amount by microscopic examination. We can also tell which genes it carries in triple amount from the trisomic ratios thrown by hybrids. In this way it is possible to identify a given chromosome of Datura as the bearer of certain genes.

Euploidy and Aneuploidy.—When a cell has exactly one set of chromosomes or any exact multiple of one set (as 2, 3, 4), it is said to be *euploid* (*eu*, good plus *ploid*, multiple). It is to be contrasted to an *aneuploid* cell (*an*, not). A $2n + 1$ type is an example of an aneuploid. But the term "aneuploid" also refers to cases in which a segment of a chromosome is present in extra amount or is deficient. Euploidy does not result in genic unbalance; aneuploidy does.

In the case of an aneuploid, such as a $2n + 1$ type, the extra chromosome can be regarded as part of a set, so that the aneuploid

in question has two sets and part of a third. It is therefore referred to sometimes as a heteroploid (hetero, different). Now genic unbalance produced by whole chromosomes is as a rule so great that development is not possible or is very abnormal. Therefore, heteroploids are not usual in nature, but they do sometimes seem to occur. In Carex (a grass-like plant of the sedge family) there are species in which $n = 28, 30, 33, 34, 35, 37, 39, 40, 42$. We do not know the exact explanation of cases of this sort. But if a chromosome consisted largely of inert material, it might be added to a normal set without causing much genic unbalance. Thus, for example, if the basic number in Carex is 28, then in the species with 30, 33, etc., the extra chromosomes are perhaps largely inert. In a certain race of corn it has actually been found that an inert chromosome might be represented ten times in each unreduced cell.

Aneuploidy has undoubtedly been of great importance in the evolution of new types, but it has been dependent largely on small deficiencies and duplications for its effects.

SUMMARY

1. In most plants and animals there are normally two chromosomes of each kind, but if by accident there should be three of one kind and two of the rest, then development would be abnormal. Any change in the normal proportions of the chromosomes to one another represents *genic unbalance*, and in general genic unbalance causes either abnormal development or failure to develop.

2. There might be a doubling in the number of chromosomes of each kind, as from two to four. Such an increase would not alter the relative proportions of the chromosomes to one another, and it would therefore not represent genic unbalance. Hence development would be normal for the most part.

3. A cell in which there are four chromosomes of every kind, or four sets, is referred to as *tetraploid*. A diploid cell (or $2n$) might give rise to a tetraploid cell (or $4n$) if chromosome division was not followed immediately by cell division. The tetraploid cell might then multiply by later cell division and produce a mass of tetraploid cells.

4. If a tetraploid cell arose in the bud of a plant, it might divide and give rise to one or more tetraploid flowers. These would produce diploid (or $2n$) gametes, and two diploid gametes might then combine to form a tetraploid offspring. From this a tetraploid race might arise.

5. Tetraploid races are often found among plants.

6. Tetraploid plants can be artificially produced by treating the buds or seeds of normal plants with the drug *colchicine*. In the treated tissue the chromosome number is doubled in an occasional cell, from which tetraploid plants can eventually be derived.

7. Tetraploid races are seldom found in species with separate sexes because (1) a male and female tetraploid would seldom arise at the same time and breed with each other, as would be necessary if they were to have tetraploid offspring and (2) even if they did breed with each other, they would produce tetraploid offspring of composition $XXXY$ (XX coming from the female, XY from the male), and such offspring would be sterile intersexes. In most animals the sexes are separate, and therefore tetraploid races are not common in the animal kingdom.

8. In Datura, a tetraploid plant with purple flowers might be hybrid for white and of genotype $\dfrac{w}{+}\bigg|\dfrac{w}{+}$. At the reduction division a combination of any two genes might go to the same pole, so that if we numbered the genes 1, 2, 3, and 4, the possible combinations would be $1\cdot2$, $1\cdot3$, $1\cdot4$, $2\cdot3$, $2\cdot4$, and $3\cdot4$. These combinations would result in four classes of gametes in the ratio of $1 + + : 4 + w : 1\,w\,w$. Since only one gamete in si s $w\,w$, only one offspring in 36 would be $\dfrac{w}{w}\bigg|\dfrac{w}{w}$. All the rest would contain one or more $+$ alleles and would be purple.

9. It is sometimes possible to cross two species of plants (A and B) and to get offspring which contain a set of chromosomes from each species ($A\,B$). If a doubling of the chromosome number now took place, it would be possible to get offspring with four sets of chromosomes, two from each species ($AABB$). Such offspring are referred to as *allo-tetraploids*, as contrasted to *auto-tetraploids*, or races with four sets of chromosomes, all of which are derived from the same species.

10. It is possible to cross the radish and the cabbage (two different species). The hybrid contains one set of chromosomes from each parent and therefore is diploid. It is very infertile, because radish and cabbage chromosomes are not sufficiently alike to attract one another and pair, with the result that the reduction divisions are very irregular. Occasionally both sets of chromosomes go to the same gamete, and when two such gametes combine, they form offspring with four sets of chromosomes, two sets being derived from the radish and two from the cabbage. These offspring are allo-tetraploids and are called *Raphano-brassica* (from *Raphanus* for radish and *Brassica* for cabbage). If we let R stand for one set of radish chromosomes and C for one set of cabbage, then they are $RRCC$.

At the reduction division R pairs with R and C with C, or $\dfrac{R\,C}{R\,C}$. Hence each

gamete gets both R and C, or one whole set of radish chromosomes and one whole set of cabbage. The union of two such gametes $(RC + RC)$ produces allo-tetraploid offspring $(RRCC)$. Hence the allo-tetraploid is perfectly fertile and breeds true.

11. Chromosome doubling might be repeated, so that a $2n$ race might give rise to a $4n$ and $4n$ to $8n$. The $2n$ race might then cross with the $4n$. An n gamete (from the $2n$ race) and a $2n$ gamete (from the $4n$ race) might combine giving an $n + 2n$ or $3n$ offspring (a triploid). In like manner, other multiples of n might be produced. Races which contain two sets of chromosomes and in addition one or more whole sets, are called *polyploids*.

12. Related plants sometimes form a series of polyploids. Thus in wheat n-7 (the original haploid number of chromosomes), and different species of wheat have the following chromosome numbers: $14(2n)$, $28(4n)$, $42(6n)$.

13. In plants an ordinary haploid egg sometimes develops without fertilization, and gives rise to a haploid plant, as in Datura. Such plants are genically balanced and normal in appearance, but they are sterile because the one set of chromosomes does not have another set with which to pair at the reduction division, and haploid plants therefore do not form normal gametes (each with a complete set of chromosomes).

14. In Datura a plant sometimes has two whole sets plus one normal chromosome (or $2n + 1$). Such a plant is called a *trisomic*. It is genically unbalanced and it shows some mutant trait as a result of its genic unbalance. There are 12 different kinds of chromosomes in Datura, and there are twelve different kinds of trisomics, each containing a different kind of chromosome in extra amount and each showing a different set of mutant traits.

15. In Datura it is possible to get $2n + 1$ plants in which the extra chromosome is made up of a doubled half of some chromosome (as $2n + 1 \cdot 1$ or $2n + 2 \cdot 2$, in which 1 and 2 are the halves of $1 \cdot 2$). Such plants are called *secondary* $2n + 1$ types in contrast to *primary*, or $2n + 1$ types in which the extra chromosome is normal (as $2n + 1 \cdot 2$). A $2n + 1 \cdot 1$ plant contains segment 1 four times (once in each $1 \cdot 2$ chromosome and twice in $1 \cdot 1$). Therefore it is more unbalanced for segment 1 than the corresponding primary $(2n + 1 \cdot 2)$, and it shows certain mutant traits in more exaggerated form than the primary.

16. In a $2n + 1$ Datura the extra chromosome sometimes is made up of halves of two different chromosomes, as $2n + 1 \cdot 18$ (the 1 half of the extra chromosome coming from $1 \cdot 2$, the 18 half from $17 \cdot 18$). Such a plant is called a *tertiary* $2n + 1$ type. It is unbalanced for halves of two different chromosomes and it contains in part the mutant traits found in two different primaries.

17. In a trisomic a certain chromosome is represented three times and all the rest only twice. If a mutant gene (as w, for white flowers) is con-

tained in the chromosome that is represented three times, then the hybrid (as $+ \mid +$) throws offspring in a trisomic ratio (35 : 1) instead of the ordinary 3 : 1 ratio. Thus we know that the gene in question is contained in the particular chromosome which is represented three times in the trisomic.

18. A cell is said to be *euploid* if it contains one set of chromosomes or any exact multiple of one set. An *aneuploid* cell is one which contains one or more kinds of chromosomes out of proportion to the rest, or which contains a deficiency or a duplication. Euploidy is the equivalent of genic balance; aneuploidy, of genic unbalance.

19. Sometimes a chromosome is inert. It therefore does not cause genic unbalance when present in extra amount.

20. Aneuploidy has been of great importance in the evolution of new types, but it has been dependent largely on small duplications for its effects, since greater unbalance is often lethal.

PROBLEMS

1. Given a diploid species with a rod-shaped and a dot-shaped chromosome in its gametes. Draw a metaphase stage of mitosis in the diploid and in the tetraploid derived from this diploid. Below each show the two products of cell division. Show also the reduction division (metaphase and telophase) in each case. (Assume that all four chromosomes of a kind come together in the tetraploid.)

2. Assume that a diploid plant hybrid at a given locus (a/A) gives rise to a tetraploid $\left(\dfrac{a \mid a}{A \mid A}\right)$ and that the four chromosomes of each kind (originally similar for the most part) become differentiated into two pairs $\left(\dfrac{a\ a}{A\ A}\right)$. Assume further that a plant with one A (or more) is of phenotype A. Given a plant of genotype $\dfrac{a\ a}{A\ A}$ (call this the F_1) and let this plant be self-fertilized. Give the F_2 abbreviated genotypic ratio (a 9 : 3 : 3 : 1 ratio) and the F_2 phenotypic ratio.

3. Like genes in different chromosome pairs (as the A's in Problem 2) are sometimes referred to as "duplicate factors." Tell through what process duplicate factors arise and how they can be detected (from what kind of an F_2 ratio).

4. Suppose that a tetraploid $\left(\dfrac{A \mid A}{A \mid A}\right)$ became differentiated into a diploid $\left(\dfrac{A\ A}{A\ A}\right)$ and that an A mutated to the recessive a. Could a very well come to express itself in any later generation? Tell why or why not.

5. Some species of plants show a much lower mutability than other closely related species. Give a possible explanation of this fact, involving a difference in chromosome number (tetraploid as compared with diploid).

6. Given two closely related species of plants (A and B), each of which has two chromosomes (a long and a short one) in its gametes. Let the two species be crossed. Show the union of the gametes to form the diploid hybrid. Draw the chromosomes of A more heavily than those of B, so as to distinguish between them, but make them otherwise similar. Show next how this diploid hybrid might give rise to an allo-tetraploid as the result of irregular distribution of the chromosomes at the reduction division. Finally, show the reduction division in the allo-tetraploid.

7. Show a second method by which the above allo-tetraploid might have arisen from the diploid hybrid.

8. If species A and B, above mentioned, each carried genes for vigor that the other lacked, how would you expect the allo-tetraploid to compare in size and vigor with either parent species from which it was derived?

9. The allo-tetraploid *Raphano-brassica* is much taller and more vigorous than either the cabbage or the radish (attaining a height of from 7 to 10 ft.). Account for this and tell by what term vigor of the sort in question is designated, as for example, when seen in corn.

10. The United States Department of Agriculture is attempting to cross related species of crop plants and to derive allo-tetraploids from the diploid hybrids. What object do you suppose they have in mind, and why should not the diploid hybrid itself suffice for this purpose?

11. Given a group of closely related plants with the following chromosome numbers in their vegetative cells: 12, 24, 48; 18, 30, 36, 42. Tell how the species with chromosome numbers above 12 originated or might have originated (giving all possibilities if there is more than one possibility, as for example in the case of a species with 36 chromosomes). Tell which of these species could reproduce only asexually and why.

12. In Datura, white flowers (w) are recessive to purple ($+$). Given a tetraploid Datura of genotype $\dfrac{w}{w}\bigg|\dfrac{+}{w}$. Tell in what ratio this plant produces its gametes. Also give the genotypic and phenotypic ratio in which its offspring are produced when it is self-fertilized.

13. The "fourth" chromosome of Drosophila is very small. A fly which is otherwise diploid might have either one fourth chromosome (a "haplo-IV" fly) or three (a "triplo-IV") and yet appear almost as normal as a fly with two fourth chromosomes (a "diplo-IV" fly). Bent wings (bt) is a mutant gene in the fourth chromosome, recessive to its normal allele ($+$). Give the results of the following crosses: (1) a diplo-IV bent $\times$ haplo-IV non-bent; (2) triplo-IV of genotype $+\bigg|\dfrac{\diagdown bt \diagup}{}+$ by diploid bent (bt/bt).

16. *MUTATION*

UTATIONS have been known for a long time and are familiar facts to the practical breeder. The short-legged race of sheep known as the Ancon breed was derived from a single short-legged sheep that cropped up in the flock of a New England farmer in the latter part of the eighteenth century. There was no previous record of such a short-legged animal in the flock in which it arose or in any neighboring flock, and it was probably a mutant. Many of the color varieties in sweet peas arose in much the same way; so did numerous other varieties of domesticated plants and animals. They took their origin from a single ancestor that was an isolated departure from the normal.

But often the practical breeder does not keep complete records of his stocks and the possibility always exists that the apparently new things which he observes are recessives and have been existing under cover in hybrids, and that he has not really observed any new mutations.

The Controlled Observation of Mutations in Drosophila. —In more recent times well-controlled observations have been made on various forms of life and new mutations definitely observed. This applies particularly to the fruit fly Drosophila. Occasional departures from the normal can be observed in Drosophila, and by the proper genetic procedure it can be shown that these sometimes represent new mutations. We must be sure that we begin with a pure stock and that we exclude hybridity as the cause of the new types. This can be done by beginning our observations with a single couple of normal flies and inbreeding their offspring. Take the recessive gene vestigial (v) in illustration. We might inadvertently have selected for the beginning of our observations two apparently normal parents but both might have been hybrids ($v/+ \times v/+$). In this case, however, one quarter of the

offspring would be vestigial in accordance with the ratio $1 +/+$ (pure normal) : 2 $v/+$ (hybrid normals) : 1 v/v (vestigial). We should then become aware of the fact that vestigial already existed in our stock at the start of our observations and we should not regard it as a new mutation. Then too we might have begun with a hybrid and a pure normal ($v/+ \times +/+$). The offspring of this mating would be 1 $v/+$ (hybrid normal) : $1 +/+$ (pure normal). All of them would appear normal, and we should not yet know that we had started with a hybrid parent. But if we inbred the offspring, making numerous cultures and using just one couple of flies per culture, then some of the cultures would contain two hybrids ($v/+ \times v/+$), and a quarter of their offspring would be vestigial. Again, we should not regard vestigial as a new mutation, since it was already in existence at the start of our observations. We should therefore have to inbreed our cultures for at least two generations before we could regard the abnormal fly as a new mutant; that is, a mutant that arose in the course of our observations.

In the case of genes in the X chromosome it would be very simple to detect recessive genes that were present at the start. A male shows any sex-linked genes which he carries, since he has only one X. A female has two X's and might be hybrid for a recessive sex-linked gene without showing it, but half of her sons would receive the recessive and would show it, and so we should be made aware that it was not a new mutation.

In Drosophila precautions have been taken for the exclusion of old recessives carried by hybrids, as just described, and a large number of new mutations have actually been observed—several hundred. Many of these mutations have been located in the chromosomes and can be found in the chromosome maps shown on pp. 194–5. But each mutation is a relatively rare event. Only about one fly in 100,000 shows a mutation of the more conspicuous kind, such as a change from red eyes to white.

The mutations found in Drosophila are not necessarily due to the artificial conditions under which they are reared in the laboratory. Many of them can be found outside the laboratory, carried by normal-appearing heterozygotes.

Mutations and Acquired Traits.—In Drosophila increased size might be due either to a mutation or to good feeding of the developing larvae. In the first instance a change in a gene some-

how makes possible the more rapid assimilation of food under ordinary cultural conditions, so that if the mutant and normal larvae are intermingled in the same mass of food, the mutants become larger. But when genetically normal flies become larger as the result of good feeding, then the offspring of the flies in question are of normal size if they are grown under normal conditions. Increased size due to feeding is referred to as an *acquired* trait. We might in general define an *acquired trait as a departure from the normal that is not due to any genetic alteration.*

The Size of Mutations.—Mutations sometimes cause very large changes, such as a change in eye color from red to white or the loss of wings in a fly. But they might also cause very small changes, so small in fact that they might escape casual observation. The eye color might become just slightly lighter than normal, the wings slightly shorter, the bristles slightly thinner, and so on.

Earlier students of mutation got the impression that mutations caused only large changes. This is to be expected from the fact that the large changes are the most conspicuous and are therefore the first to attract attention. But the impression in question was due in large measure to another fact that can be explained in connection with a concrete example. Suppose that a mutation produced just a small size difference instead of a large one. Then even though it happened to be noticed, it would not be *recognized* as a mutation. For small size differences are always occurring in any stock of animals or plants regardless of mutation. They are small acquired traits. They appear frequently even if the stock is pure and is grown under substantially uniform conditions. Thus if a small mutation did occur, it would look no different from the numerous small acquired traits that are constantly occurring.

It is possible to detect small mutations by special methods, and it has been found that small mutations greatly outnumber the large. When mutations are classified as to size, they form a continuous "spectrum" ranging from those that are so small that they are hardly detectable to those that are very large.

The Sporadic Nature of Mutations.—When flies are reared in the laboratory they usually are kept in milk bottles with banana as food. The conditions within a given bottle are probably pretty much the same for all the flies; still a mutation might take place in just one fly in the bottle, not in any of the rest. Moreover, even in the one fly the mutation is confined when it first occurs to

a single cell and to a single gene within that cell. Not even the allele changes as a rule, though it is the same kind of gene as the one that mutated and is located within the microscopic confines of the same cell! The mutation is *sporadic*—isolated and unpredictable.

The factors which cause mutations are themselves microscopic in dimension and so might influence one minute part of a cell such as a gene without influencing the rest of the cell or anything else in the surroundings. One cause of mutations seems to be the thermal agitation of molecules or parts of molecules, similar to that which often causes chemical changes in general.

The Spreading of a New Mutation.—Mutations are not necessarily confined to the sperm or egg cells. They might take place in any cells, and at any stage of development. But a mutation could be transmitted to the offspring of a fly only in case it occurred in the germ track; that is, in a sperm or egg cell or in a cell ancestral to a sperm or egg cell. When a mutation arises early in the germ track of a fly, it is multiplied by cell division and may go to numerous sperm or egg cells, the number depending on how early the mutation has arisen. For example, let us say that the germ track is in the sixteen-cell stage and that the mutation vestigial arises in a cell of the germ track at this stage, and in a male. Then by cell division about one-sixteenth of the unreduced cells of the mature testis will come to contain the mutated gene vestigial. But these cells would be hybrid for vestigial $(v/+)$, since only one allele mutated in the original cell. Hence only half of the sperm cells formed by the unreduced sixteenth in the mature testis would receive vestigial; that is, one thirty-second of the total sperm cells. A corresponding proportion of the offspring would then receive the mutation.

The First Appearance of a New Mutation.—If a dominant mutation occurred in a given parent it would of course show in any of the offspring that received it. It might show up in any proportion of the offspring, depending on how early it arose in the germ track of the parent. Likewise, sex-linked mutations would express themselves immediately in any males which received them, since the male has only one X.

But most mutations are recessive and are not in the X; that is to say, they are *autosomal recessives*. These might not express themselves for several generations after their first occurrence. Thus

assume that a mutation to vestigial (an autosomal recessive) has taken place in a single sperm cell of a fly and that there are as yet no other flies in existence with the mutation. The fly that carries the mutation must therefore mate with a pure normal, and the union of the mutant sperm cell with a normal egg would produce a normal-appearing hybrid $(v/+)$. But the F_1 hybrid in turn can mate only with pure normals (or F_1 $v/+$ $\times$ $+/+$), and this cross produces F_2 offspring in the ratio of 1 $+/+$: 1 $v/+$. All of the F_2 appear normal, though half of them are hybrid. Thus the mutation has not appeared even in the second generation after its occurrence. But if the second generation offspring were bred in isolated couples (just one couple being used per culture bottle), then as a matter of chance two hybrids might sometimes be placed in the same bottle and these then would produce third generation offspring in the ratio of three normals to one vestigial— the Mendelian ratio. The effect of the mutation therefore has not shown up until three generations after its initial occurrence.

If the mutation to vestigial had taken place in an early cell of the germ track of a given parent, then more than one F_1 offspring might have received the mutation. These would be hybrids $(v/+)$, since only one parent carried the mutation. If the F_1 were bred in isolated couples, then by chance two hybrids might be brought together $(v/+ \times v/+)$ and they would produce F_2 in the ratio of 3 normal : 1 vestigial. But unless the F_1 were isolated in single couples (individual cultures), the normal and mutant would not at first appear in the 3 : 1 Mendelian ratio. For the F_1 are a mixture of hybrids and pure normals, and if the hybrids and normals were bred in the same bottle (in a mass culture), then only occasionally might two hybrids happen to mate, and under the crowded conditions in the bottle they might produce just one vestigial offspring. Thus the new mutant would have appeared as a sporadic isolated fly among a large number of normals, instead of in the 3 : 1 Mendelian ratio as earlier explained. The mutation itself did, however, arise as an isolated sporadic change in the original ancestor, and so it just happens that its sporadic appearance in mass culture conforms to its sporadic occurrence in an earlier generation. In a state of nature there would often be crowding and a recessive mutation would as a rule make its first appearance in an isolated individual.

We might, then, summarize as follows. If a mutation is a domi-

nant or is sex linked, it might show up immediately in the offspring of the parent in which it occurred, and in almost any ratio to the normal. If on the other hand a mutation is an autosomal recessive and if it occurs in an isolated sperm or egg cell (of the adult) it will not appear earlier than the third generation following the ancestor in which it first occurred. If it occurs early in the germ track, it might make its first appearance in the second generation. When individual matings are made (matings of one male and one female per culture), the mutation makes its first appearance in the 3 : 1 Mendelian ratio. In mass cultures (or mass populations) an autosomal recessive mutation does not as a rule make its first appearance in the Mendelian ratio. Instead it often appears as an isolated mutant.

Mosaics and Bud Sports.—A dominant mutation might show itself directly in the parent in which it occurred, provided it occurred sufficiently early in development. So might a sex-linked mutation if it occurred early in a male. Thus suppose that the normal allele of yellow (which is in the X) mutated to yellow in a cell of a developing male fly and that this cell gave rise to a patch of skin cells. These particular cells would then be of composition $y/$, and they would be yellow. The size of the patch would vary with the time in development when the mutation arose—the earlier the mutation, the larger the number of cells to which it would be transmitted and the larger the patch.

In the fly just referred to the skin cells outside the yellow patch would have the normal (unmutated) allele of yellow and would have the normal gray color. In other words, the fly in question would be gray with a yellow patch. Such flies have been observed and are referred to as *mosaics*. This term is applied to any animal or plant which has a visibly spotted distribution of a newly arisen mutation. The spotted coat color of some breeds of dogs is not due to a newly arisen mutant gene. It is due to a gene which is present in all the skin cells but which simply expresses itself in an irregular, spotted manner. Some people have one brown and one blue eye. We do not know exactly why. It is possible that they are hybrid for brown and blue ($b/+$) and that early in development the normal allele for brown ($+$) mutated to blue (b) in one eye, making it pure for blue (b/b). On the other hand, it is possible that the normal allele (for brown) simply did not express itself in the one eye, and that the eye is still $b/+$.

In plants a mutation might arise in a cell from which a twig is later derived by cell division. If the mutation were a dominant, the twig would show the mutation and the plant with the aberrant twig would be a mosaic. The mutation could then be reproduced by means of cuttings, but now the entire plant would be mutant tissue, not just a twig. Dominant mutations have arisen in twig buds of fruit trees and have given rise to new varieties, such as the nectarine peach and the naval orange. They are known to the breeder as *bud sports*.

The Germ Track and Mutations.—Some biologists are of the opinion that the cells of the germ track are more susceptible to mutation than cells in other parts of the body and that this would apply in particular to germ cells which are undergoing the reduction division. It is possible that irregularities connected with crossing over might occasionally lead to mutations, but this sort of thing would by no means be the usual cause of mutations. For one thing, mutations are just about as frequent in the male of Drosophila as in the female, though there is no crossing over in the male. Also the study of mosaics and of bud sports in plants shows that mutations do occur outside the germ track and in these cases are in no way connected with the reduction division or crossing over.

Lethals.—A mutation which seriously interfered with the development of some vital organ, say, the heart, would cause the death of the developing embryo. It would be a *lethal*. There are many genes which cooperate in the development of vital organs, and since mutations often are adverse changes, a high proportion of them interfere with vital organs and are fatal.

Lethals like other mutations might be dominant or recessive. If a dominant lethal arose, say, in a sperm cell of Drosophila, it would not destroy the fly in which it arose since it would be limited to just the one sperm cell. But any egg which that sperm cell fertilized would be incapable of developing to the adult stage, even though the egg itself contained the normal allele, since the lethal is dominant. Thus dominant lethals would die off almost as quickly as they arose and we should not be aware of their existence. A recessive lethal, however, might continue its existence in a hybrid. Thus if we let l stand for the lethal and $+$ for the normal allele from which it arose, then the hybrid would be $l/+$;

and since $+$ is dominant to l, the hybrid would be capable of normal development, like a pure normal.

It will be recalled that in Drosophila recessive lethals in the X chromosome cause a disturbance in the sex ratio. This is seen when a female hybrid for a sex-linked lethal $(l/+)$ is mated to a normal male $(+/\ )$. Half the sons receive the lethal from their mother and die in accordance with the formula $l/+$ (hybrid mother) $\times$ $+/$ (normal father) gives $1+/+$ (pure normal daughter) : 1 $l/+$ (hybrid normal daughter) : 1 $+/$ (normal son) : 1 $l/$ (lethal-containing son, which dies in development). Lethals in the X of Drosophila can therefore be detected by the disturbance they produce in the sex ratio ($2\ \female : 1\ \male$).

Most of the genes of Drosophila are not in the X but in the autosomes and so most lethal mutations would be in the autosomes. Since the autosomes are paired in both sexes, a male as well as a female might be hybrid for an autosomal lethal $(l/+)$ and the mating of two hybrids $(l/+ \times l/+)$ would yield offspring in the ratio of $1\ +/+ : 2\ l/+ : 1\ l/l$. The pure lethals (l/l), both male and female, would fail to develop. The adult offspring would all be normal in appearance, and there would be equal numbers of males and females among them. Therefore they would not tell us that they carried a lethal. There would be fewer offspring than ordinarily expected, but a shortage of offspring would in itself not necessarily indicate a lethal; it might, for instance, be due to poor food. But autosomal lethals can be detected by special methods.

Sometimes an egg that is pure for a lethal develops for a while and gives rise to a visibly defective embryo which can then be seen to die. This for example is true in certain races of mice and chickens which carry lethal mutations.

The Yellow Mouse Case.—A mutant gene might have both a visible effect and a lethal effect. A case of this sort was early discovered in mice by Cuenot. The ordinary or normal race of mice is gray but there is a yellow race which arose from the gray race by a mutation of one of the normal genes for coat color. The mutant gene yellow (Y) is dominant to its normal allele $(+)$. Whenever yellow mice are bred to one another they not only produce yellow offspring but also some grays in the ratio of 2 yellows : 1 gray. In other words, yellow mice are never pure.

We could account for the above fact on the assumption that the yellow mutation has a recessive lethal effect. That is to say, the

yellow mutation has two effects, one a dominant effect on coat color and the other a recessive lethal effect. Because of the lethal effect, a pure yellow mouse (Y/Y) dies early in development. But the hybrids $(Y/+)$ can live because the normal allele $(+)$ suppresses the recessive lethal effect of the Y. Thus all yellow mice are necessarily hybrids. When two hybrids interbreed $(Y/+ \times Y/+)$, they produce embryos in the usual Mendelian ratio, which in this case is $1\ Y/Y : 2\ Y/+ : 1\ +/+$. Since the Y/Y die as embryos, the ratio among the offspring that survive is $2\ Y/+ : 1\ +/+$, or 2 yellows to 1 normal (gray).

Dominant mutations having a recessive lethal effect are not unusual. In Drosophila several such mutations have been found, including Beaded (scalloped wings), Truncate (tips of wings cut off), Star (rough eyes), Dichaete (absence of certain bristles), and Hairy wing.

Ever-sporting Varieties.—Some genes are much more mutable than others. One of the earliest cases of a highly mutable gene was worked out by Correns in the flowering plant Mirabilis (the "four-o'clock"). There is a certain variety of Mirabilis that has white flowers with blotches and streaks of red; that is to say, it has variegated flowers. The red areas are due to mutations. The variegated race is basically white, and it probably arose from the normal red-flowered race by a recessive mutation which changed the flowers to white. But the mutant gene is unstable and is constantly reverting to the normal allele $+$ (red). We can therefore refer to the mutant gene as unstable white (u). A variegated plant starts out pure for unstable white (u/u) but as it develops, a u occasionally reverts to $+$. This might happen in one of the cells of a flower bud and result in a cell of genotype $+/u$ (from the original (u/u). The cell in question might then divide and give rise to a patch of cells (all $+/u$) on a petal of a flower. The normal allele $(+)$ is dominant and the patch would therefore be red. If the remaining cells of the petal had not changed and were still u/u (white), we should have a white petal with a patch of red; that is, a variegated petal. Several red patches might of course arise on the same petal provided u changed to $+$ in several cells of the originally white flower bud (u/u). The size of a patch depends on how early the mutation of u to $+$ arises in the development of the bud. Sometimes the whole flower is red, and in this case the mutation to $+$ must have arisen not later than when the

flower bud was represented by just one cell. It might happen even earlier, in a bud of a twig that gives rise to several flowers, all of which would then be completely red.

When a variegated flower is self-pollinated, the offspring are usually variegated, but some might be red. If both pollen and eggs happen to be in a white area, then both are of composition u, and when they combine they produce a fertilized egg of composition u/u. This grows into a variegated plant. However, some of the gametes of a variegated flower might be included in a red area. Since a red area is of genotype $+/u$, half the sperm and egg cells in this area are $+$ and half u. Therefore, self-pollination of a red flower or red area would produce offspring in the ratio of $1 +/+$ (pure red); $2 +/u$ (hybrid red); $1 u/u$ (variegated).

Although the white gene of the variegated race of four-o'clocks mutates frequently compared to other genes, yet even it is fairly stable. This can be seen from a rough calculation. Let us assume that about a third of a variegated flower is red and that the same proportion of genetically red tissue occurs over the plant as a whole. Therefore a white gene in about one cell in three mutated during the development of the plant, and since each cell has two white genes at the start, this would amount to one white gene in six. Allowing four months for the development of the plant, the mutation rate becomes one gene in six per four months, which amounts to the same thing as one mutation per two years per gene. The white gene is therefore still fairly stable.

The variegated race of four-o'clocks is sometimes referred to as an *ever-sporting* variety (the term "sport" being the practical breeder's expression for a mutant). Other flowering plants besides four-o'clocks have ever-sporting varieties. In Portulaca and Delphinium there are races with irregularly striped flowers, the stripes again being due to mutation of a gene with a relatively high mutation rate. The red stripes found on the kernels of a certain variety of corn are due to the same thing.

One must, however, be on his guard in assuming that stripes in plants are always due to mutations. We cannot be sure that they are in any particular instance until we have got seeds from within and from without the striped areas and found that they grow into differently colored plants. However, when the stripes show great variations in size there is the suspicion that they may be due to a mutating gene, for stripes so produced would vary greatly

in size in accordance with the time in development that the gene mutated.

Dotted Aleurone in Corn.—Figure 130 shows the ear of a race of corn known as "Dotted aleurone," discovered by M. M. Rhoades. Each dot represents a mutation. Originally, the plant on which the kernel grew was pure for the recessive gene albino (*a*) which causes the aleurone to be white instead of colored as normal. Ordinarily the gene *a* is very stable, so that a plant pure
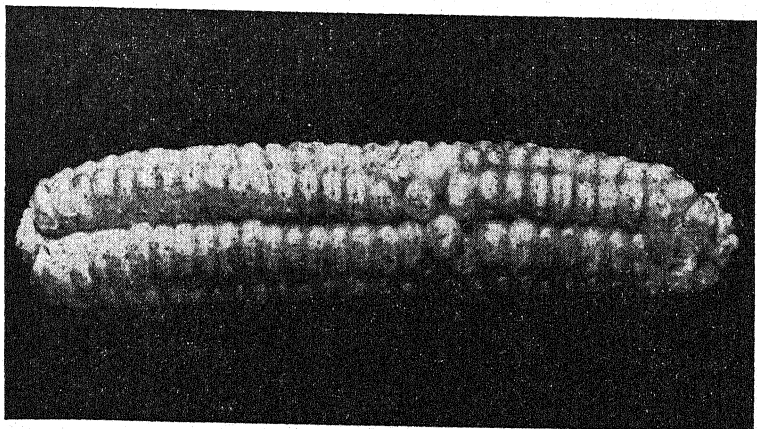
Fɪɢ. 130. Dotted aleurone. Each dot represents a mutation. (From M. M. Rhoades in *Genetics*.)

for *a* would have pure white kernels. But in the race discovered by Rhoades there is a mutant gene (entirely distinct from *a*) which causes *a* to mutate rather frequently to its normal allele (+), for pigment, and each dot is derived from a single cell in which an *a* has mutated to its normal allele. The mutant gene which causes *a* to be unstable is eventually causing the corn to be dotted, and it is this gene (rather than *a*) which is called "Dotted aleurone" (*Dt*, a dominant). It has no effect on the stability of any gene other than *a*, so far as is known.

Rhoades' case suggests that other cases of the same sort occur in both plants and animals. Perhaps, indeed, it is not unusual for a mutation in a given gene to influence the mutability of some other gene.

Unstable Genes in Drosophila virilis.—In *Drosophila virilis* Demerec has found that a fly might sometimes have one normal

wing and one miniature (Fig. 131). Miniature (mt) is in the X chromosome of *D. virilis* and is recessive to its normal allele ($+$) for long wings. The fly shown in Fig. 131 is a mosaic. It was originally miniature (mt/mt if female, $mt/$ if male). The long wing was derived from a cell in which a miniature gene (mt) mutated, early in development, to its normal allele, so that the long wing was derived from a cell which changed from mt/mt to $+/mt$ in case the mosaic was a female, and from $mt/$ to $+/$

in case the miniature was a male. If the mutation to long wings had occurred later in development, then just half the wing might have been normal, the rest miniature; or, if the mutation had occurred still later, it might have caused just a small island of wing tissue to be normal. The two kinds of wing tissue—normal and miniature—are somewhat different in texture, so that a

FIG. 131. A mosaic fly (*Drosophila virilis*). The long wing (right) arose by mutation, the fly originally having been pure for miniature alpha (an unstable gene for the short type of wing shown on the left side of fly). (From M. Demerec in *Zeit. f. Ind. Abs. u. Ver.*)

small island of normal tissue can be identified upon close examination of the wing with a lens.

Demerec found that the miniature wing might be of five distinct sizes, labeled consecutively mt-1 to mt-5 in the order of their discovery; but in the order of their size (beginning with the smallest) they are mt-1, mt-3, mt-2, mt-5, and mt-4. Two of the sizes (mt-3 and mt-5) are each represented by three different genes differing in stability but not in their effect on wing size. The three different genes for size mt-3 have been labeled mt-3 alpha, beta, and gamma; and the three for size mt-5 have been labeled mt-5 alpha, beta, and gamma. The remaining three sizes (mt-1, mt-2, mt-4) are each represented by only one gene (labeled respectively mt-1, mt-2, mt-4). In the case of mt-3 and mt-5, the alpha genes are unstable in both the body and germ cells in the sense that they frequently mutate back to the normal allele ($+$); the beta genes do not mutate in either the body or germ cells; and the gamma genes mutate back to normal in the body cells but are stable in the germ cells. Thus if the alleles are arranged in a horizontal line according

to increasing size of wings and down a vertical line according to increasing degree of instability, we have:

mt-1———mt-3 beta———mt-2———mt-5 beta———mt-4

(stable)   (fairly stable)   (stable)   (fairly stable)   (stable)

mt-3 gamma mt-5 gamma

(unstable in body cells)   (unstable in body cells)

mt-3 alpha mt-5 alpha

(unstable in body and germ cells)   (unstable in body and germ cells)
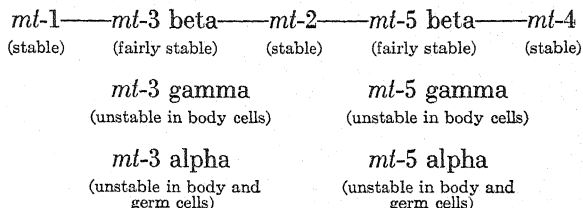
A gene for one miniature size, say, mt-1, does not mutate to one for another size, mt-2; the genes for each size originate independently, though very rarely, from the normal allele. But one of the genes for size mt-3 occasionally mutates to another for size mt-3; and likewise for genes of size mt-5.

Miniature alpha flies ordinarily produce three kinds of offspring: (1) miniature alpha, (2) normals, and (3) mosaics. The miniature alphas (1) do not contain the normal allele (they are pure for miniature alpha), and of course no reverse mutation to normal could have taken place, either in the germ cells from which they were derived or in their own somatic cells. The normals (2) are due to germinal mutations in the parents; the mosaics (3) to somatic mutation in the developing offspring themselves. In one instance miniature alpha parents produced the following number of offspring: 1,531 miniature alpha; 2,487 long; 2,211 mosaic. In this particular example the reverse mutations to normal are fairly frequent, and the high frequency is partly due to a dominant gene (M) which stimulates the mutability of miniature alpha. But it has an effect only on the germinal mutability of miniature alpha, not on its somatic mutability. The reason for all this is not known.

Miniature gamma flies produce no normal offspring, since the miniature gamma gene does not mutate to the normal allele in their germ cells. They produce only miniatures and mosaics. All the offspring are originally pure miniature, but in some of them a miniature gene mutates to its normal allele in one or more body cells during development, and thus the mosaics arise. The mutability of miniature gamma is increased by at least three genes, one of which for example might increase the per cent of mosaics in a stock from 5 per cent to 95 per cent.

In addition to the genes at the miniature locus, Demerec has found two other mutable genes in *Drosophila virilis*, one for magenta eye color and the other for reddish body. But apart from the genes at these three loci in *Drosophila virilis*, no other cases of mutable genes are known in the animal kingdom. By contrast, several dozen mutable genes are known in the plant kingdom.

Differences in Mutation Rates.—Genes in the same organism show relatively great differences in their mutation rates. In corn Stadler studied the mutation rates of seven genes which influence the endosperm (the part of the seed which surrounds the germ). Corn seeds were got by fertilizing a plant pure for recessive mutant genes with pollen from plants pure for the normal alleles. If a normal allele mutated to the recessive allele, then the seed which got the mutation would be pure for the recessive and would therefore give evidence of the mutation. One of the genes investigated was *R* (Table 6). This is one of the normal genes for purple aleurone, dominant to *r* for colorless aleurone. The number of gametes tested for mutations of *R* to *r* was 554,786 (Table 6, second column, top), and in this number there were 273 mutations (of *R* to *r*), amounting to an average mutation rate of 492 mutations per million gametes. Another gene investigated was *Wx* (Table 6, column one, bottom). This is one of the normal genes for starchy endosperm, dominant to *wx* for waxy endosperm. In this case 1,503,744 gametes were tested, without the detection of a single mutation of *Wx* to *wx*. Thus Stadler's results show that some genes are relatively mutable, others very stable; and between these two extremes there are all intermediate degrees of mutability (Table 6).

TABLE 6. DIFFERENCES IN MUTATION RATES OF GENES IN CORN
(FROM STADLER)

Gene	Gametes Tested	Number of Mutations	Average per One Million Gametes
R...........	554,786	273	492
I...........	265,391	28	106
Pr..........	647,102	7	11
Su..........	1,678,736	4	2.4
Y...........	1,745,280	4	2.2
Sh.........	2,469,285	3	1.2
Wx.........	1,503,744	0	0

Certain mutant genes might increase the mutation rate of other genes, as clearly shown in Rhoades' and in Demerec's cases mentioned above. This is not so surprising if one bears in mind the fact that a mutated gene might conceivably cause the production of some agent, say, an acid, which might perhaps produce mutations. In corn, Beadle has found a mutation ("sticky") which makes the chromosomes sticky and greatly raises the frequency of mutations of all kinds.

The mutation rate as found in Drosophila melanogaster might vary greatly from one experiment to another, depending on the particular stock of flies used. Thus the rate at which sex-linked lethals occur has been found to vary from one lethal in about two hundred X's per generation to less than one in a thousand. Undoubtedly, the observed differences are due to mutant genes which themselves influence the mutation rate and which differ from one stock to the other.

Some species seldom mutate, as judged by the fact that they have so few varieties. This, for example, is true of the goose. By contrast there are many varieties of chickens, due perhaps in part to a relatively higher mutation rate. Closely related species might differ markedly in their mutation rate. In plants polyploidy would tend to suppress the expression of recessive mutations, since a polyploid contains several sets of chromosomes and a recessive would show only if it were contained in each set. A tetraploid race, for example, might show a much lower mutation rate than a closely related diploid race.

Muller has pointed out that a gene which increased the mutation rate, say, in Drosophila, would among other things increase the rate for the X chromosome and would result in defective males (since most mutations cause defects) and it would tend to eliminate itself through its immediate effect on the X. Hence the larger the X the more rapidly would such a gene be eliminated and the lower would be the mutation rate. Preliminary reports of various authors indicate in fact that *Drosophila pseudo-obscura*, with its double-size X, has about half the mutation rate of *D. melanogaster*.

Reverse Mutations.—Sometimes a mutant reverts to normal. This is known as "reverse" mutation. In Drosophila the mutant known as "forked bristles" sometimes reverts to the normal under X-ray treatment; so does eosin (an eye color mutation at the

white locus). The rate with which reverse mutations occur varies greatly from one mutant to another. In the case of certain unstable mutants (such as alpha miniature), the return rate might be very high. But usually a mutant very seldom reverts to the normal.

Are All Mutations Due to Chromosome Breakage?—It will be recalled that in Drosophila duplications and deletions might have mutant effects. The possibility therefore arises that sometimes a duplication or a deletion might be too small to be visible under the microscope but that it might nevertheless have a mutant effect. There would then be no way of distinguishing between mutant effects due to actual alteration of individual genes (point mutations) and those due to invisibly small duplications and deletions. Then, too, an inversion might be too small to be visible, yet it might have a position effect. Now deletions, duplications, and inversions are all the result of chromosome breakage. Therefore, in the present state of our knowledge, all mutant effects might conceivably be due to chromosome breakage, not to any actual alteration in the gene itself. It might seem that perhaps reverse mutations have some bearing on the problem. If, for example, forked bristles were due merely to a loss, then the reverse mutation to normal would have to be a gain, and so all mutations could not be losses, as sometimes claimed. But it is possible that the normal "allele" of forked is a minute duplication or "repeat" (say, $a\ a$) and that forked is a deletion (a instead of $a\ a$). A duplication might then produce a reverse mutation to normal ($a\ a$). We therefore should have no evidence for an actual "point" mutation—a qualitative change in a gene itself.

It is not likely, however, that all mutations are due merely to chromosome breakage or losses, for if they were then new kinds of genes could not have come into existence in the course of evolution, and we should be driven to the absurd conclusion that a man evolved from an Ameba simply by rearrangements and losses. This would make man genetically less complex than Ameba. Obviously new kinds of genes must have come into existence in the course of evolution, and this could have happened only through point mutations—actual changes in the genes themselves.

The Classification of Mutations.—The term mutation, when used in its broadest sense, means any hereditary change not due to Mendelian segregation and recombination. Mutations in this sense can be classified as follows:

I. Changes in genes, or *point* mutations.
II. Changes in chromosome number, due to
  1. Increase or decrease in the number of chromosome sets (polyploidy and haploidy).
  2. The addition or subtraction of less than a whole set of chromosomes (heteroploidy).
III. Changes in the arrangement of chromosome segments, due to
  1. Intra-chromosomal segmental rearrangement (inversions).
  2. Inter-chromosomal segmental rearrangement (translocations).
  3. Losses and duplications of chromosome segments (deletions and duplications).

When we speak of mutations, we usually have gene changes or point mutations in mind. The other kinds of mutations result in an increase or decrease in the number of genes in a cell, or in a change in their arrangement, but in no change in kind.

The Fundamental Problem of Mutation.—Just how a gene changes into some other kind of gene is not known. Perhaps the most remarkable thing in connection with mutation is the fact that a gene, after having mutated, should reproduce itself in its *changed* form. How this can happen is one of the fundamental problems of biology. It is really remarkable, too, that a change originating within the microscopic dimensions of a single cell should be capable of spreading until it covers the earth with some new form of life.

SUMMARY

1. By the term "mutation" is meant any relatively permanent change in the germ plasm other than one that results from Mendelian recombination. The term as ordinarily used means a change in a gene.

2. Mutations have been known for a long time. Varieties of flower color in sweet peas and other flowering plants are due to mutations or recombinations of mutations. Most other varietal differences in domesticated plants and animals are due to the same cause.

3. An apparently new mutant might sometimes be due to the coming together of recessive genes carried by hybrids for many generations. Hence, before a mutant can be regarded as evidence of a *new* mutation, it must be established that the mutant gene is not already present in the stock that is under observation.

4. In Drosophila mutant races have been observed to arise in stocks known to be free of the mutant genes at the start of the observations. Several hundred new mutations have been observed and located in the chromosomes. But each individual mutation is very rare, as a rule. In the case of most genes, less than one in 100,000 mutates during the entire life cycle of a fly.

5. Mutations might cause changes of all sizes ranging from very small to very large. In Drosophila, for example, a mutation might cause the eyes (normally red) to become only slightly lighter than normal; or it might cause them to lose all their pigment and become white.

6. A mutation of a given kind as a rule does not take place in more than one cell of a given individual. It may then spread by cell division to other cells. But often it is confined to one or a few cells, and then it has little or no visible effect on the individual in which it arises.

7. The number of sperm or egg cells that receive a mutant gene depends on how early the mutation arises in the germ track. The earlier it arises, the greater the number of sperm or egg cells that receive it.

8. A dominant mutation expresses itself in any offspring that receive it, the ratio of mutants to normals depending on how early the mutation arises in the germ track of the parent. A sex-linked mutation which arises in a female fly expresses itself in any of the male offspring which receive it, since a male has only one X.

9. If a mutation is an autosomal recessive, it does not express itself in the F_1 but might in the F_2 if several F_1 offspring receive it and happen to breed together. Normals and mutants would then be produced in the 3 : 1 ratio. But in "mass cultures" the F_1 would often breed with pure normals and only occasionally would two of the F_1 produce a mutant.

10. A fly sometimes contains a patch of mutant tissue in an otherwise normal body. It might, for example, contain a patch of yellow skin in an otherwise gray body, or a fleck of white in an otherwise red eye. Such flies are called "mosaics." They are usually males and the mutant tissue is usually due to a mutation in the X chromosome, the cell in which the mutation occurred having produced the mutant tissue by cell division. The mutation expresses itself immediately in the mutant tissue, even though it is recessive, because a male contains only one X. An autosomal mutation, if dominant, might also express itself immediately and produce a mosaic.

11. In plants new varieties sometimes arise as dominant mutations in buds and are known as "bud sports." The nectarine peach and navel orange are bud sports.

12. Mutations are not confined to the germ track, for in mosaics mutant tissue is often derived from cells outside the germ track. Mutations might sometimes be due to irregularities in crossing over, but this is not the usual cause of mutations, for the mutation rate is not greatly reduced by an absence of crossing over.

13. Lethals are mutations which kill the developing embryo. In Drosophila they occur in all the chromosomes. They might be either dominant or recessive.

14. In mice, yellow is a recessive lethal, but it is dominant for its effect on coat color, yellow being dominant to gray (the normal coat color). Pure yellow mice cannot develop to maturity because of the lethal effect of yellow. When two hybrids interbreed ($Y/+ \times Y/+$), they produce offspring in the ratio of 1 Y/Y : 2 $Y/+$: 1 $+/+$. The pure yellow (Y/Y) die as embryos. Hence the ratio among the offspring that survive is 2 $Y/+$: 1 $+/+$, or 2 yellows : 1 gray.

15. In Mirabilis (the four-o'clock) there is variegated variety that has white flowers with blotches and streaks of red. Each red area is due to a mutation of white to red. A variegated plant is originally pure for unstable white (u/u). Each red area is derived from a cell in which one u mutated to the normal allele for red ($+$, dominant to u). The red areas therefore are $+/u$.

16. In corn albino aleurone (a) is ordinarily very stable, but a mutant gene called "Dotted aleurone" causes albino to mutate rather frequently to purple ($+$) and so causes an albino ear to contain numerous purple dots, each derived by cell division from a cell in which a mutated to $+$.

17. In *Drosophila virilis* the locus of miniature wings contains a number of alleles for miniature wings. Two of these (designated as miniature alpha genes) mutate rather frequently to long wings ($+$) in both the germ cells and the body cells. Two others (designated as miniature gamma genes) mutate rather frequently to long wings in the body cells but not in the germ cells. The mutation frequency of both kinds of miniature is greatly increased by mutant genes at other loci.

18. In corn different genes show considerable differences in their mutation rates, mutant genes of different kinds ranging in number from about one in every 2,000 gametes to less than one in a million.

19. In Drosophila the lethal mutation rate in the X chromosome varies greatly in different stocks, ranging from about one lethal in two hundred X's per generation to less than one in a thousand. Such differences are undoubtedly due to mutant genes which differ from one stock to another and which stimulate the mutation rate. The mutant genes in question do not necessarily have any visible effect of their own.

20. Sometimes a mutant gene mutates back to its normal allele, as when forked bristles in Drosophila mutates to non-forked. This is known as *reverse mutation*.

21. Mutations can be classified as (1) changes in genes, or point mutations, (2) changes in chromosome number (resulting in polyploidy, haploidy, and heteroploidy), (3) changes in the arrangement of the chromatin (translocations, inversions, deletions, and duplications).

22. Only gene mutations can result in new kinds of genes. These therefore are the most important class of mutations in evolution.

23. Fundamentally, evolution is possible because a gene can change and reproduce itself in its changed form.

PROBLEMS

1. In Drosophila the body is normally gray. Black body (*b*) is a mutation in the second chromosome, recessive to its normal allele (+). Given a pure gray fly (+/+), say, a male, and assume that a mutation occurs in a single cell of his germ track (that is, in a sperm cell or a cell ancestral to a sperm cell). Assume further that if the mutation occurs in an unreduced cell, only one of the two normal alleles in the cell mutates to *b* (that is, +/+ becomes *b*/+ in the cell in question).

a. Tell how many sperm cells would receive the mutated gene (1) if the mutation occurred at the second meiotic division (the one that produces the cells that differentiate into the sperm cells, otherwise known as the equation division or second maturation division), (2) if the mutation occurred at the first meiotic division (before the splitting of the paired chromosomes), (3) just before the division immediately preceding the second meiotic, (4) just before the one next earlier.

b. Would the mutation express itself in the fly in which it arose? Tell why or why not. Would it express itself in the immediate offspring of the fly in which it arose? Tell why or why not. (Assume that at the start no flies carry the mutation except the one in which it arose.)

c. Assume that a mutation to black occurs rather early in the germ track of a pure gray male, so that more than one sperm cell gets the mutation. Give the genotype of the flies that receive the mutation in the first generation (the F_1). Assume that two such flies happen to mate. Give the genotypic and phenotypic ratio in which their offspring (the F_2) would be produced.

d. Would all F_1 matings produce the mutants in the F_2 in the ratio you just worked out? Tell why or why not. Give the formulas for all the possible matings and tell which of these would produce the mutants in the F_2. How would the proportion of such right matings vary with the period of development at which the mutation occurred in the parent?

e. Drosophilas can be grown in milk bottles (supplied with banana for food), either in "mass culture" (numerous parents in one bottle) or in individual matings (just one couple in a bottle). If the first generation that received the black mutation were mated in mass culture, would the mutation probably appear in the next generation in any definite ratio? Give your reason, as follows: Consider first how the ratio would vary in accordance with the number of flies that received the mutation in the first genera-

tion, and second with the number of flies that received the mutation and that happened to mate with each other.

2. In Drosophila the eyes are normally red. White (w) is a mutation in the X chromosome, recessive to its normal allele ($+$). Given a female that is pure red at the start ($+/+$). Assume that a mutation to white occurs in one of the early cells of the germ track of this female. Assume further that this female is bred to a red-eyed male ($+/$).

a. Give the genotype and phenotype (1) of the female offspring that receive the mutation, (2) of the male offspring that receive it, (3) of all the rest of the offspring.

b. The number of mutated genes discovered in the X chromosome of Drosophila is greater than the number in an autosome (of like size). Does it follow that the genes in the X chromosome mutate more frequently than those in autosomes? Give your reason (in view of your answer to the question in paragraph **a** above).

3. Given a Drosophila that is pure gray at the start. Assume that a mutation to black (an autosomal recessive) takes place in a cell outside of the germ track.

a. Could the mutant gene (black) be transmitted to the offspring?

b. If the mutant gene arose in the embryo in a cell ancestral to some skin cells, might it be transmitted to a patch of skin cells? If so, how? Would it make this patch of cells black? Why or why not?

c. If the mutation to black had been dominant, what would have been the color of the patch of cells (referred to in paragraph **b**) that received it?

d. At what stage in development would a mutation have to occur if it were to be transmitted to all the unreduced cells of the animal in which it arose?

4. In Drosophila yellow body (y) is recessive to gray ($+$) and is located in the X chromosome. Given two flies, both normal at the start, one a female ($+/+$) and the other a male ($+/$). Assume that a mutation to yellow occurs in each fly, and that it is transmitted (by cell division) to a patch of skin cells. Tell how the patch would differ in appearance in the male and the female, and give the reason for the difference, if any.

5. In Drosophila, males have been found with one eye white, one red (mosaics). Account for the fact that only one eye was white, if a mutation occurred, and tell why the mutation was able to express itself, even though it was a recessive. Also tell whether the mutation (a) might have been transmitted to some of the offspring of the male in question; (b) whether it necessarily was. Give your reason.

6. Suppose you crossed a pure gray fly to a black ($+/+ \times b/b$) and that one of the offspring was black. Tell why this would indicate that a mutation to black had probably occurred in the gray parent.

7. Suppose you X-rayed some normal male flies and wanted to see whether you produced recessive mutations of certain kinds, say, any one or

more of the following autosomal recessives: black (body), purple (eyes), vestigial (wings). To what would you cross the treated males in order to detect, in the F_1, mutations of the kind in question (black, purple, vestigial), and what would you look for in the F_1? What would be the appearance of all F_1 flies not carrying mutations at the loci under discussion?

8. New varieties of plants which arise as "bud sports" are always due to dominant mutations. Explain why.

9. The unstable white gene which mutates to the stable red in four-o'clocks is sometimes referred to as the gene for variegation. Is the unstable gene itself really producing variegation (both white and red)? If all the cells of a flower were pure for the unstable gene, what would be the color of the flower?

10. Spotting might be due either to a stable gene with irregular expression or to the combined effects of an unstable gene and a stable mutation to which it gives rise. Given a plant with spotted flowers, the normal being unspotted. How would you determine to which of the above-mentioned causes the spotting was due?

17. *THE ARTIFICIAL PRODUCTION OF MUTATIONS*

WHAT causes mutations? One way of attacking this problem is to treat animals or plants with some agent that might be suspected of producing mutations, such as heat, and to see whether or not the agent in question actually does produce them. Until rather recently the causes of mutations were unknown. But in 1927 H. J. Muller showed that mutations could be artificially produced. Muller treated flies with X-rays and produced many different kinds of mutations—white eyes, vestigial wings, forked bristles, and in fact almost all of the ordinary run of mutations in Drosophila. Subsequent investigators have shown that X-rays produce mutations in corn, barley, cotton, mice, and many other forms of life.

X-rays undoubtedly produce mutations in man, and their therapeutic use will have to take account of this fact, particularly since most mutations are changes for the worse. The hereditary effects produced by X-rays would not necessarily be noticed in one generation, for they might be recessive. But they might show up in a later generation, especially if there were inbreeding. The small exposures to X-rays employed in X-ray photography probably do no harm, if not repeated too often. But persons who work around X-ray machines might be subject to dangerous doses (over a long period) unless they took precautions to shield themselves from the X-rays.

Lethals as a Measure of the Mutation Rate.—When we say that X-rays produce white eyes, we do not mean that all the offspring of a treated fly have white eyes. The vast majority are still red-eyed. Now, white eyes and other mutations sometimes occur in the absence of X-ray treatment, and to prove that X-rays produce mutations it is necessary to know not only the mutation

rate in treated material but also in untreated material, or controls. We should then have to compare the rate in treated and controls to see whether or not treatment *increased* the rate. If it did we could conclude that the treatment produced mutations, since the mutations represented by the increase would have been produced by the treatment. But mutations are of many different kinds. In general, mutations may be classified according to whether they produce (1) *visible* changes such as red to white eyes or (2) *lethal* effects.

Visible mutations might be large in the sense that they might cause a conspicuous change, as, for example, a change from red to white eyes. But they might also be small, as when they cause only a slight change in the shade of the eye color. Now an observer who was looking for visible mutations might detect more small visibles on one occasion than on another, depending on his condition, and so the rate at which he observed them to occur would vary. He could not very well get around this difficulty by ignoring the slight visibles and confining his observations to the large ones, for there is no sharp gap between large visibles and small, and the observer would therefore not know where to draw the line between them. When on the other hand lethals are used for gauging the mutation rate, the error due to the personal element is not so great, for there is usually a sharp distinction between lethals and non-lethals. Hence lethals are an accurate index of the mutation rate.

The *ClB* Method.—X-rays produce lethals in all the chromosomes of Drosophila, but we shall confine our discussion to just those produced in the X chromosome. There is a standard technique for the detecting of new lethals in the X chromosome of Drosophila devised by Muller and known as the *ClB* method (Fig. 132). It involves the use of females in which one X contains a crossover suppressor (C), a recessive lethal (l), and the dominant gene Bar eyes (B). The other X is normal. Such flies are known as *ClB* females. Males are treated with X-rays and mated to *ClB* females. The Bar daughters contain the *ClB* chromosome (derived from their mother) and also the treated X (derived from their father). They are crossed to any male. Half their sons (F_2) receive the *ClB* chromosome and die. The other half receive the treated X and they also die if the treated X contains a lethal. But all the F_2 daughters survive (since they receive a normal X from

their father, "any male"). Hence, if an F_1 *ClB* female produces daughters but no sons, we know that she must have received a lethal from her treated father.
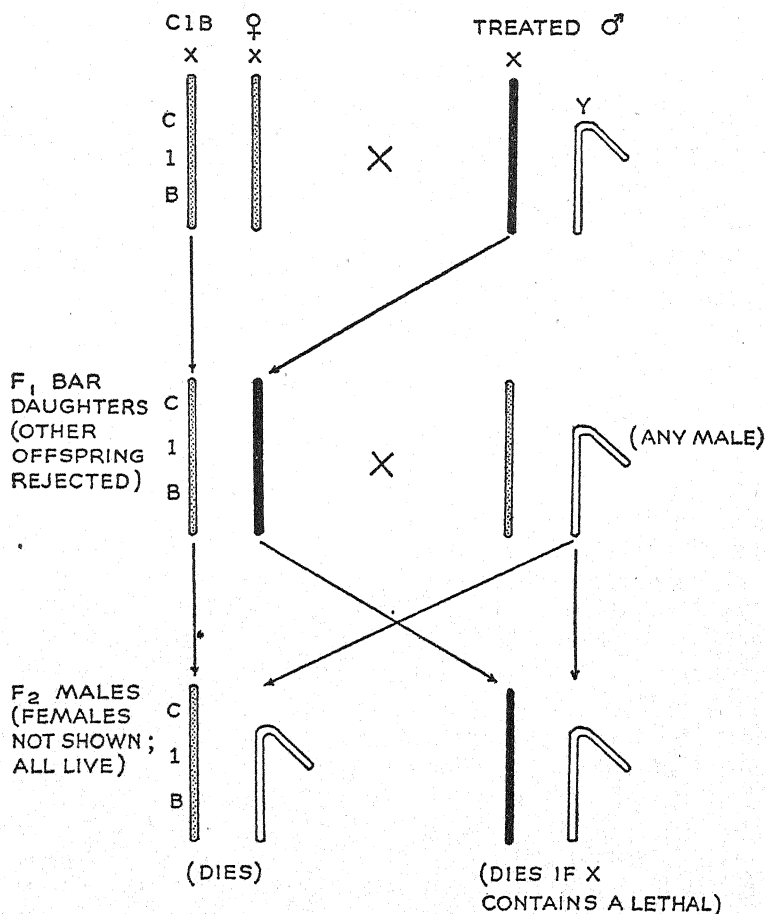
Fig. 132. The *ClB* method for detecting lethals.

We could not be positive that the new lethal was produced by the treatment the father received, because it might have arisen spontaneously (apart from the treatment). But if we had run two series of experiments, one in which males were treated and another in which they were not (or treated and controls), and if we found that there was a significantly larger number of lethals in the

treated series as compared with the controls, then we could conclude that the treatment was producing lethal mutations. In order to get significant figures we should have to rear a great many ClB females in the F_1—several thousand in both the treated and control series. Each female would have to be reared in a separate bottle or vial in order to determine whether or not she produced any males.

If the treated X had contained a visible mutation (such as white eyes), then it would show in the F_2 sons that received the treated X. Hence the ClB method can be used for detecting visible mutations as well as lethals.

It is possible for a mutation to arise early in the germ track of a male, and for the mutation then to become multiplied through cell division. It might thus become distributed to a good many sperm cells and to a corresponding number of daughters. But it would represent only a single mutation. We can count two mutations as two only if we know that they are of *independent* origin. Now, in an adult male Drosophila many sperm cells are formed and in store. Moreover, it takes from a week to ten days for new sperm cells to mature. Therefore when an adult male is treated with X-rays and bred within a week or ten days, the treatment is in effect restricted to the mature sperm cells, and all mutations that are detected must be of independent origin (since the mature sperm cells do not multiply).

The Effect of X-rays on the Mutation Rate.—The effect of X-rays on the mutation rate is dependent on the dosage. It was found by Muller in his first work that the greater the dosage used the greater was the effect. An exact study of the relation of dosage to effect was then made by Oliver working under Muller's direction and using the ClB method. It turned out that the effect of X-rays on the rate of lethal mutations in the X chromosome is directly proportional to the dosage. This was shown by first taking five different samples from a mixture of flies that were grown under the same conditions and that were substantially the same in every respect. The five samples were then given five different dosages of X-rays. A sixth sample was untreated and served as the control. The smallest dosage used in the treated series was a 3½-minute treatment with X-rays of a certain intensity, this dosage being represented as t_1 in Table 7; t_2 is double t_1 (or 7-minute treatment); and so forth. The controls are represented as

C. Males were treated and mated to *ClB* females, as usual for the *ClB* method. The F_1 *ClB* females contained the treated X of their father, and they were bred to see whether or not their paternal X contained a lethal. Their offspring represent the F_2 cultures in Table 7. In the case of the sample given the t_1 treatment, 4,016 F_2 cultures were reared, and of these 57 were found to contain sex-linked lethals, or 1.42 per cent. This per cent represents for the most part the lethals produced by the treatment, but a small part is due to lethal mutations that would have arisen

TABLE 7. THE DIRECT PROPORTIONALITY OF THE RATE OF LETHAL MUTATIONS INDUCED BY X-RAYS TO THE DOSAGE OF THE X-RAYS

Dosage	Total Number F_2 Cultures	Observed Number of Lethals	Per Cent of Observed Lethals	Per Cent of Observed Lethals Due to Treatment
t_1........	4,016	57	1.42	1.18 ± 0.135
t_2........	2,231	72	3.23	2.99 ± 0.256
t_4........	1,144	55	4.90	4.56 ± 0.428
t_8........	618	61	9.87	9.63 ± 0.74
t_{16}......	435	70	16.09	15.85 ± 1.19
C........	4,033	10	.24	0

even if the treatment had not been given, and this part is considered as equal to 0.24, the per cent of lethals observed in the untreated material or controls (C), so that the per cent of lethals produced by the t_1 treatment is equal to 1.42 (the total per cent observed) less 0.24 (the per cent in the controls) or 1.18 (the ± 0.135 means that the "true" per cent based on a much larger number of F_2 cultures would probably not be more than 0.135 above or below 1.42 per cent). Inspection of Table 7 will show, then, that for t_1 the per cent of lethals produced by the treatment is 1.18; for t_2 it is 2.99, or somewhat over two times 1.18; for t_4 it is 4.56, or about four times 1.18; and so on. In short, when the dosage of X-rays is doubled, the per cent of lethals produced by the X-rays is doubled. Hence the effect of the X-rays on the lethal mutation rate is directly proportional to their dosage.

Weak X-rays produce mutations, and if they act over a long enough period they produce the same number of mutations as stronger X-rays over a shorter period, provided the dosages are the same, just as weak light of long duration causes as much

darkening of a photographic plate as stronger light over a shorter period, provided the total "dosage" of the light is the same in the two cases. Moreover, the mutation rate is independent of the wave lengths of the X-rays used, provided again the dosage is kept constant. (X-rays vary in wave length from about 1 A to 10 A. The letter "A" denotes a minute unit of length, an Angstrom unit, equal to one hundred millionth of a centimeter, a unit used in expressing the length of light waves, X-rays, and other forms of radiation.)

X-rays are a very efficient agent for producing chromosome breakage, and therefore they can readily produce all classes of rearrangements dependent on breakage; namely, translocations, inversions, deletions, and duplications. But all these rearrangements require two breaks, and therefore two "hits" by the X-rays. As a result the per cent of the rearrangements produced is proportional to the *square* of the dosage of the X-rays (previously pointed out). But in the case of point mutations only one hit is required per mutation and therefore the rate of induced point mutations is directly proportional to the dosage.

FIG. 133. The attached-X method for detecting visible mutations in the X chromosome of Drosophila.

The Attached-X Method. —A special technique is sometimes used to detect visible mutations, known as the "attached-X" method. It will be recalled that in Drosophila there is a race in which the females carry attached-X's and also a Y chromosome. In the attached-X method normal males are treated with X-rays and mated to attached-X females (Fig. 133). The sons then receive any visible mutations which the treated X might contain. Many of the smaller visibles would of course escape detection.

The Production of Visible Mutations by X-rays.—Muller was able to produce practically all the different kinds of visible mutations that were observed in untreated material by all Dro-

sophila workers combined over a period of seventeen years. This he was able to do by X-ray treatments amounting in the aggregate to a total of a few hours. In one experiment Muller treated the adult males and mated them to untreated females with attached-X's. The sons, of course, got the treated X of their father in this mating. The total number of sons observed was 2,344, and of this number, 12 had visible mutations of one kind or another, in the X (Table 8). The mutations were all visibles of the more conspicuous kind (such as yellow body instead of gray), and the

TABLE 8. THE PRODUCTION OF VISIBLE MUTATIONS IN THE X CHROMOSOME OF DROSOPHILA BY X-RAYS. MALES WERE TREATED AND MATED TO UNTREATED FEMALES WITH ATTACHED-X'S, AND THE F_1 MALES WERE EXAMINED FOR VISIBLE MUTATIONS IN THE X

Dosage	Number of F_1	Number of F_1 Males with Visible Mutations in the X	Rate
t_8	1,724	8	1 in 215
t_{16}	620	4	1 in 155
Total	2,344	12	1 in 195

number observed (12 in a count of 2,344 flies) amounts to one visible sex-linked mutation in 195 flies, a rate for conspicuous visibles far greater than that ever observed in untreated material. No controls are shown in Table 8, for if the same number of controls had been observed as the number treated (2,344) it is improbable that any visible mutations at all (of the more conspicuous kind) would have been observed in the controls.

It is difficult accurately to measure the increase in the rate of visible mutations produced by X-rays, because of the personal factor previously mentioned, which plays such an important part in detecting visible mutations, especially those slight visibles that are on the borderline between the visible and invisible and which are relatively frequent.

The Manner in Which X-rays Produce Mutations.—How do X-rays produce mutations? When an X-ray photon (the smallest divisible amount of X-radiation) strikes an atom, it knocks an electron (a negative particle) out of the atom. The X-ray photon

imparts great speed to the electron, which in turn knocks "secondary electrons" out of atoms in its path, in contrast to the "primary" electron which was knocked out by the photon itself. The primary electron knocks secondary electrons out of about one atom in a thousand in its path, and each time it does so it loses some of its energy. Eventually it comes to a stop, but before doing so it produces a great many secondary electrons. When an atom loses an electron, it becomes positively charged and is said to be *ionized*. The atom is now chemically reactive, and it might cause a mutation through its chemical activity, providing it is in a gene or within chemical striking distance of a gene.

In general, any form of radiation which can produce ions would be expected to produce mutations, now that we know that X-rays do so. This has in fact been found to be true. Radium gives off three forms of radiation (alpha, beta and gamma radiation). The alpha and beta radiation is stopped almost completely by a rather thin layer of silver but the gamma radiation is hardly stopped at all, and when radium is in a silver container it gives off mostly gamma radiation through the wall of the container. Gamma radiation is very similar to X-radiation but is of still shorter wave length, and it produces ions upon striking atoms. It would be expected to produce mutations, and Hanson has in fact found that the gamma rays of radium produce mutations. Included also among the forms of radiation which produce ions are alpha particles and neutrons, and these, too, produce mutations (as shown by the author's students and co-workers, Ward for alpha particles and Nagai and Locker for neutrons).

Extent of Spontaneous Mutations Due to Radiation.— Mutations which occur apart from any special treatment are sometimes referred to as "spontaneous" mutations. When Muller found that X-rays produce mutations he raised the possibility that radiation similar to X-rays might be the cause of all spontaneous mutations. X-rays do not occur in a state of nature, but gamma rays are constantly being produced by radium and to a minor extent by other radioactive substances. Radium is contained in a kind of rock known as pitchblende, and in certain parts of the world pitchblende is relatively abundant. But there is less than a thousandth of an ounce of radium in several tons of pitchblende, and so pitchblende does not produce gamma rays in very appreciable intensity.

Rays are also being constantly showered down upon us from the outside universe, known as *cosmic rays*. These are even more penetrating than X-rays and gamma rays. But cosmic rays are extremely dilute. Comparatively few strike the reproductive organs of even a larger animal or plant per second and still fewer are absorbed.

To what extent, then, do gamma rays and cosmic rays account for spontaneous mutations? It is possible to figure out the answer to this question. Gamma rays produce about the same per cent of mutations per unit dose as do X-rays, and probably cosmic rays also do. The intensity of both forms of radiation is known, and from this it is possible to calculate the dosage which a fly is receiving in a state of nature in a given time. From this in turn it is possible to figure out what per cent of mutations this dosage is producing (on the assumption that the per cent is proportional to the dosage). Calculations of Muller and Mott-Smith show that the dosage is only enough to account for a small fraction of spontaneous mutations (less than 0.1 per cent). Factors other than short-wave radiation must therefore be causing most spontaneous mutations. We shall now consider some of these other factors.

Ultraviolet Light.—In a somewhat different category from X-rays is ultraviolet light. A photon of ultraviolet light contains just about enough energy to dislodge an electron from some kinds of atoms, as carbon and nitrogen, especially when these elements are in particular combinations. But an ultraviolet photon does not impart much speed to the electron, and so does not cause much secondary ionization. Thus the effect of an ultraviolet photon is very localized; it is limited to one atom.

Ultraviolet light can have another effect on an atom besides dislodging an electron from it. It can knock out an electron from one "shell" of the atom to another farther out. This, too, happens preferentially with atoms in given combinations. The atom is then in an unstable condition and is chemically reactive. It is said to be "sensitized." The condition of unstable equilibrium lasts for only a small fraction of a second, but during this time the atom is more likely than usual to enter into chemical reactions.

Ultraviolet light has very weak penetrating power. It cannot penetrate the surface layers of a normal adult fly and reach the reproductive organs unless it is used in very heavy doses and

then it causes grave injury to the body of the fly. However, at a certain stage in the development of a Drosophila egg the germ track cells are formed and are close to the surface of the egg (at one end, where they form the "polar cap"). Ultraviolet light can reach the germ track cells at this stage without appreciably harming the body, especially if the treatment is limited to the germ cells by shielding the rest of the egg from the ultraviolet light, as done by Geigy. The writer has found that ultraviolet light is very effective in producing mutations in Drosophila, if the egg is treated at the stage under discussion.

Muller and MacKenzie have shown that ultraviolet light produces few chromosome breakages large enough to be detectable by ordinary linkage tests or by salivary gland examination. In this respect it differs from X-rays.

The ultraviolet region of the spectrum extends from about 1,800 A to 3,800 A (to the left of the visible spectrum). It is therefore a mixture of photons of different wave lengths, and the problem arises as to just which wave lengths cause mutations. Before this problem can be attacked, the photons of different wave lengths must be separated from one another. This can be done by means of a quartz prism which spreads apart the ultraviolet spectrum in the same way as a prism of ordinary glass spreads apart the spectrum of visible light. The pollen of plants is especially adapted to experiments with ultraviolet light, for pollen cells can be spread out on a glass slide or other surface in a layer of single thickness and so can be directly exposed to ultraviolet light of a given wave length, which can then readily penetrate the thin layer of protoplasm that surrounds the nucleus of a pollen cell and reach the nucleus. Experiments of Stadler on the pollen of corn show that the regions of the ultraviolet spectrum ranging from 1,800 A to 3,100 A produce mutations (the regions that also produce sunburn), but not those above 3,100 A. Moreover, certain wave lengths within the effective range are much more efficient than others. Thus the region at about 2,600 A is more efficient than the wave lengths on either side of it. This corresponds with the fact that the wave lengths of this region are especially highly absorbed by nucleic acid, one of the important chemical constituents of the nucleus.

The Effect of Heat and Chemicals on the Mutation Rate. —Heat speeds up chemical reactions, and it might therefore be

expected to speed up the mutation rate. Some experimental evidence by Muller and the author in 1919 indicated but did not prove conclusively that heat produces mutations. However, evidence got since by Muller, Timofeeff, Goldschmidt, Jollos, and Plough has proved this point. The amount of effect obtained is far less than with X-rays, yet if the heat treatment is continued over the entire life cycle of Drosophila, the frequency of mutations produced may be increased as much as four or five times for a rise of temperature of 10° C., within the temperature range in which the fly normally lives. Abnormally high (and possible abnormally low) temperatures appear to give stronger effects in a given time, yet as these temperatures cannot be long endured, the total effect produced by this means remains rather limited.

Attempts have been made to produce mutations by means of chemicals, but most of the experiments reported to date have been negative or inconclusive. It is difficult to find a chemical which can get to the nucleus and change the genes within it without first doing grave damage to the surrounding cytoplasm and killing the cell. But recently Robson and Auerbach have found that mustard gas is a very efficient agent in producing mutations. Mustard gas and X-rays both have an irritating effect on the skin, and perhaps the one produces mutations in the same way as the other.

Radiation and Genetics.—The discovery that X-rays can produce mutations marked a new era in genetics. The cause of mutations had been one of the outstanding problems of biology, and so long as this problem was untouched it was still possible to claim that mutation, and hence evolution, had some hidden mystical basis. Once it was shown that X-rays could produce mutations it was apparent that other agents might likewise do so and that the causes of mutations and evolution were amenable to scientific attack. Moreover a tool was now available for an attack on many problems in genetics proper. Once the gene could be changed at will, then the nature of mutation itself could be studied much more effectively than before. There was now some hope of learning something of the intimate nature of the gene by changing it one way or another, just as chemists get to learn something about a compound by changing it in various ways.

X-rays also proved to be very efficient in breaking up chromosomes as a whole. This opened up tremendous possibilities in various directions. It became possible to cut up and dissect a

chromosome and to test the properties of the various parts. Thus the properties of the so-called inert or heterochromatic regions were determined by Muller, aided first by Painter and then by Prokofyeva and others, and followed later by Schultz. It was also proved that the spindle fiber attachment points and the chromosome ends are distinctive structures, the peculiar properties of which depend upon the genes present in the neighborhood of these structures.

New light was shed on the study of sex. By breaking up the *X* chromosome into fragments of various sizes, the effect of each fragment on sex could be studied and so each part of the *X* could be analyzed. Thus it was shown that there was not just one gene for sex in the *X* chromosome of Drosophila but many, distributed at random along the length of the *X*.

Then again, it was found that when a chromosome was broken by X-rays the broken ends, unlike the intact ends, were "sticky" in that any two broken ends tended to unite with one another with perfect healing at their place of union. All abnormal changes in gene arrangement occur in this way, regardless of whether they are translocations, inversions, deletions, duplications, or attached-*X* formation. Which kind of these formations arises is a matter that depends merely upon which chromosomes happen to get broken and where, and which broken ends happen to come into effective contact. But naturally the likelihood of such contacts between one kind of chromosome region and another will vary with the state of extension and the general form of the chromosomes, and this in turn varies with the condition of the cell—whether it is in the resting condition or dividing, or whether its nuclear material is highly condensed as in the sperm cell head, or less condensed as in ordinary active cells.

Very important was the part that the X-ray discovery played in the construction of cytogenetic maps. These could never have been constructed without the aid of deletions, inversions, and translocations, and these in turn could not have been got in the necessary quantity if there had not been an artificial method producing them.

X-rays have become an indispensable tool of the geneticist, and almost every large genetics laboratory throughout the world now has an X-ray machine. The study of genetics through X-rays and other forms of radiation has come to be a science in itself.

SUMMARY

1. X-rays produce mutations of all kinds in Drosophila and other forms of life.

2. In measuring the exact effect of X-rays on the mutation rate, lethals are looked for, because there is usually a very distinct difference between lethals and non-lethals, and therefore the observed lethal rate does not vary with the keenness of the observer. The opposite is true of "visibles," because some observers might see very small visibles which might escape other observers.

3. The effect of X-rays on the rate of lethal mutations in the X chromosome of Drosophila is usually determined by means of the ClB method. Males are treated with X-rays and bred to females, one X of which contains a crossover suppressor (C), a lethal (l), and the dominant gene Bar eyes (B), the other X being normal ($+$). If we let $+(t)$ stand for the treated X, the cross is $\frac{Cl\,B}{+}$ ♀ $\times \frac{+(t)}{}$ ♂. Half the F_1 daughters are $\frac{ClB}{+(t)}$. They appear Bar. These Bar daughters are bred to any male $\left(\frac{ClB}{+(t)} \text{ ♀ } \times \frac{+}{} \text{ ♂} \right)$. Half of their sons get their ClB chromosomes and die (because of the l). The other half get the $X(t)$ chromosome, and they also die if $X(t)$ contains a lethal. But all the F_2 daughters live because they receive a normal X from their father. Thus, if the treated X contains a lethal, then the F_1 ClB females produce daughters but no sons in the F_2.

4. A given dose of X-rays causes a certain per cent of lethals; twice the dose causes twice the per cent, etc. In other words, the effect of X-rays on the lethal mutation rate is directly proportional to the dosage.

5. If the dosage is kept the same, the effect of X-rays on the lethal mutation rate is independent of (1) their wave length; (2) their intensity. X-ray treatment might be either continuous or interrupted without effect on the per cent of the lethals produced, provided the dosage is kept constant.

6. X-rays readily produce breakages, and therefore they readily produce translocations, inversions, deletions, and duplications. But all these rearrangements require two independent breaks, each requiring a separate "hit" and therefore the per cent of rearrangements is proportional to the *square* of the dosage. Lethals by contrast require only one hit, and so the per cent of lethals induced by X-rays is *directly* proportional to the dosage.

7. The "attached-X" method is usually employed in determining the effect of X-rays on the per cent of mutations with *visible* effects (such as white eyes, yellow body, etc.). Males are treated and bred to attached-X females. The sons then receive the treated X of their father, and they show directly any visible mutations which their X might contain.

8. Small visible mutations can easily be overlooked, and so it is difficult to determine accurately how the per cent of visibles varies with the dosage of X-rays; but the per cent seems to be roughly proportional to the dosage.

9. X-rays produce mutations by causing "ionization."

10. There are natural rays similar to X-rays, including gamma rays (given off by radium) and cosmic rays (coming to earth from the outside universe), but the natural rays are not of sufficient dosage to account for more than a fraction of one per cent of spontaneous mutations (or mutations not induced by artificial treatment).

11. Ultraviolet light produces point mutations (both lethals and visibles), but it produces few or no translocations and no large inversions.

12. Ultraviolet light of a certain wave length (2,600 A) is highly absorbed by nucleic acid and is more efficient in producing mutations than ultraviolet light of other wave lengths.

13. Heat produces mutations. A rise of 10° C. over the entire life cycle of a fly increases the mutation rate about four or five times. Heat probably causes mutations by increasing the thermal agitation of molecules, thus causing them to bombard one another and combine.

14. Most of the harmful chemicals do not produce observable mutations because they destroy the cell before they get through the cytoplasm and reach the genes. But recently mustard gas has been found to produce mutations. This chemical is similar to X-rays in its irritating effects on the skin.

15. The discovery that X-rays produce mutations marked a new era in genetics. It proved for the first time that mutations had natural causes, and it made possible an attack on many problems in genetics, including (1) the mutation problem and the nature of the gene, (2) the nature of the chromatin (its differentiation into euchromatin and heterochromatin), (3) the nature of the centromeres and the chromosome ends (their dependence on special genes in their neighborhood), (4) the genetical basis of sex (its dependence on genes distributed throughout all the chromosomes), (5) the manner in which translocations, inversions, deletions, and duplications are produced (by chromosome breakage followed by the accidental union of the sticky, broken ends), (6) the construction of cytogenetic maps (made possible by X-rays because they require for their construction large numbers of deletions, translocations and inversions, all of which are most efficiently produced by X-rays).

PROBLEMS

1a. A normal male Drosophila is treated with X-rays and bred to a *ClB* female. Make a chromosome diagram of the parents and the F_1 similar to that shown in Fig. 132 on p. 357, but include all the F_1 classes. Show next which class of females is selected and bred, and to what kind of a

male she is bred. Make chromosome diagrams of the F_2 and tell how we could determine from the F_2 whether or not the treated X contained a lethal.

b. Tell why an F_1 *ClB* female with a lethal in her treated X lives, though she has a lethal in each X.

2. Assume, in the *ClB* method, that an F_1 non-*ClB* female contained a lethal in one of her X's. Could we tell by breeding her that the lethal in question was in the treated X? Tell why or why not. Also would there be a total absence of males among the offspring of a non-*ClB* F_1 female, assuming she did contain a lethal in her treated X? Why or why not?

3. In the *ClB* method tell what purpose is served by (1) the marker B (Bar eye), (2) the little crossover "factor" (C), (3) the lethal (l).

4. Assume that we treated a normal male Drosophila with X-rays and bred him to a normal female. Tell which of his offspring would receive his treated X. Suppose these offspring were bred to any male. Then what would be the sex ratio in the next generation or F_2 (1) if the treated X did not contain a lethal and (2) if it did?

5. Suppose that one of the F_1 females in Problem 4 (a daughter of a treated male) produced, say, 207 females and 102 males. Would her treated X probably have contained a lethal? Why? Suppose, on the other hand, she had produced only 20 females and 10 males. Would you say that it was probable or doubtful that she contained a lethal in one of her X's? What is the sex ratio? Would the sex ratio in this case be significant?

6. In an actual experiment, female Drosophilas often produce relatively few offspring (less than 30). Tell why it would be impractical to use the 2 : 1 sex ratio as a method of detecting lethals.

7. Suppose an F_1 *ClB* female (the daughter of a treated male) produced only 6 offspring, and one of these was a male. Could you be reasonably certain that her treated X did not contain a lethal? Tell why.

8a. Suppose you treated mice with over-doses of alcohol in order to see whether you could produce mutations. If the alcohol actually did produce mutations (we do not know that it does), would necessarily all the F_1 offspring, or a large proportion of them, contain mutations? Even if some did contain mutations, would they necessarily show them? Tell why or why not.

b. If some of the offspring did happen to be abnormal, would this in itself prove that the alcohol produced mutations? Tell why or why not.

9. When an animal or plant is treated with X-rays, most of the X-ray photons pass right through its cells without being absorbed, and they do no damage (the photon being small enough to pass between the molecules of the cell without touching them). The few photons which actually "strike" do very localized damage. Why would you expect X-rays to be much more efficient in producing mutations than a chemical agent which is absorbed by all parts of the cell and does widespread damage?

10. Assume that a *dominant* lethal arises in the X chromosome early in the germ track of a male Drosophila and that half the X-containing sperm cells of the adult come to contain the lethal. Assume further that the male in question is bred to (1) a normal female, (2) an attached-X female. Tell what the sex ratio would be among the offspring in each case.

11. A barley plant usually consists of about a half dozen stems arising from the ground ("tillers"), each having its origin from a separate cell in the seed. Stadler X-rayed normal barley seeds and he found that the adult plants grown from the seeds sometimes contained a tiller which (upon self-fertilization) produced offspring of its own in the ratio of 3 normals : 1 mutant type (such as white seedlings instead of the normal green), the remaining tillers producing normal offspring. Account for these results.

18. *BALANCED LETHALS AND CHROMOSOME COMPLEXES*

$\bigcup$SUALLY when a stock is uniform from one generation to the next it is pure, not hybrid. But there are exceptions to this rule. The evening primrose, *Oenothera lamarckiana*, is hybrid; yet it breeds substantially true from one generation to the next. However, it does occasionally throw aberrant offspring. These were at one time considered mutants, but it was later discovered that they were the result of hybridity, and so they can be referred to as *spurious mutants*. We shall now consider the explanation of this and similar cases.

Balanced Lethals.—Mutations sometimes have a dominant visible effect and a recessive lethal effect, as in the case of the yellow mouse. One of the earliest instances of this kind found in Drosophila was "Beaded," a mutation which causes indentations in the margins of the wings, leaving beadlike remnants of the margin in between the indentations. A fly which is pure for Beaded (Bd/Bd) cannot develop because of the lethal effect of Bd. But if a fly is hybrid for Beaded ($Bd/+$) it can develop because the normal allele ($+$) prevents Bd from exerting its recessive lethal effect. At the same time the hybrid appears beaded because of the dominant visible effect of Bd.

Given now a fly which has Beaded (Bd) in one chromosome and an ordinary recessive lethal (1) at some other locus in the homologous chromosome. We can represent this fly by the formula $\dfrac{Bd\ +}{+\ l}$ (where Bd stands for the Beaded gene, l for a lethal, and the $+$ signs for the normal alleles). Assume further that the $+\ l$ chromosome contains an inversion which acts as a crossover suppressor (C) between Bd and l. The formula of the fly then becomes $\dfrac{Bd\ \ +}{+\ C\ l.}$

This fly forms just two classes of gametes (1) $Bd +$ and (2) $+ C\,l$. When two such flies mate, sperm cells of class (1) might fertilize egg cells of either class (1) or (2), and so might sperm cells of class (2). This would give us three expected classes of offspring in the ratio of $1 \dfrac{Bd \quad +}{Bd \quad +} : 2 \dfrac{Bd \quad l}{+ C\,l} : 1 \dfrac{+ C\,l}{+ C\,l}$. The first class would die because it is pure for beaded (Bd), and the third class would die because it is pure for the ordinary lethal (l). But the second class $\left(\dfrac{Bd \quad +}{+ C \quad l}\right)$ would live because the normal alleles prevent Bd and l from exerting their lethal effects. In brief, then, only the heterozygous offspring would live. These are of the same genotype as their parents and upon mating they produce the same three classes of offspring as their parents. Again only the heterozygous offspring live, and so the stock is of constant composition from generation to generation in spite of the fact that it is hybrid. It is 100 per cent beaded.

Offspring pure for either lethal cannot live, but the lethals by themselves would not make the stock heterozygous and constant. For suppose the stock did not have the crossover suppressor and were simply of composition $\dfrac{Bd +}{+\ l}$. Then by crossing over the upper $+$ and the l in the above formula would sometimes exchange positions, giving two classes of crossovers, namely, (1) $Bd\,l$ and (2) $+ +$. These would be formed in the female. In the male there would be no crossing over but half the sperm cells would be of class $+\,l$, and if one of these fertilized an egg cell of class $+ +$ (the second crossover class given above), then an offspring would be formed of genotype $\dfrac{+ +}{+\ l}$. This would be capable of living and would be normal in appearance. But the crossover suppressor prevents eggs of class $+ +$ from being formed, and so prevents the occurrence of the normal-appearing offspring.

The constancy of the Beaded stock is due, then, to two things: (1) the presence of a lethal at a different locus in each chromosome of the pair under consideration and (2) the crossover suppressor which prevents crossovers from coming through. We might think of the two chromosomes of the heterozygote as the two pans of a balance, and the two lethals (Bd and l) could be likened to two

equal weights, one on each pan. Thus the two lethals are balanced from one generation to the next, and we can refer to them as *balanced lethals*. The stock is forced to remain heterozygous and is therefore said to be in a state of *enforced heterozygosis*. This state is brought about through balanced lethals.

Enforced heterozygosis was discovered by Muller, and the theory of balanced lethals was first advanced by him in explanation of this discovery.

Spurious Mutations.—Beaded flies ordinarily contain no further mutant genes than those already mentioned and are of genotype $\dfrac{Bd \quad +}{+ \quad C \; l}$. But a beaded fly might be got which contains the recessive mutant gene p (pink eyes) in the $Bd +$ chromosome and the normal allele of p in the $+ C l$ chromosome. The genotype of the fly then becomes $\dfrac{Bd \quad + \; p}{+ \quad C \; l \; +}$. This fly would have the normal eye color (red) because p is recessive to its normal allele.

The gene p is not included within the limits of the inversion (C) and so crossovers can be formed between the inversion and p. But p is close to the inversion, and so there is very little crossing over between l (which is included in the inversion) and p. In a male, all of the gametes are non-crossovers over the entire length of the chromosome, since there is no crossing over at all in the male. In a female the vast majority of the gametes also are non-crossovers, even between l and p. Therefore when two flies mate, each of genotype $\dfrac{Bd \quad + \; p}{+ \quad C \; l +}$, the viable offspring are for the most part also of this genotype. They show no evidence of the pink eye gene, since they contain the normal allele of pink $(+)$. But in the female parent occasional crossing over takes place between l and p, so that in the above female $\left(\dfrac{Bd \quad + \; p}{+ \quad C \; l \; +}\right)$, p and its normal allele exchange positions $\left(\dfrac{Bd \quad + \; +}{+ \quad C \; l \; p}\right)$. As a result of this a few eggs are formed of class $Bd + +$ and $+ C l p$. An egg of the second class $(+ C l p)$ might now be fertilized by a sperm cell of class $Bd + p$ $\left(\text{from a male of genotype } \dfrac{Bd + p}{+ C \, l +}\right)$. This combination

would result in an offspring of genotype $\dfrac{+\ C\ l\ p}{Bd\quad +\ p}$. It would live because it has the normal alleles of Bd and l. At the same time it would be pure for p and therefore would have *pink* eyes. But most of the offspring are of genotype $\dfrac{Bd\quad +\ p}{+\ C\ l +}$. They are heter-ozygous for pink and therefore have normal (red) eyes. The pink-eyed fly is thus an isolated exception or variant among a larger number of offspring with normal eye color (red), and if we had not known the history of the stock from which it came, we might have pronounced the pink-eyed fly as a mutant.

In brief, the gene for pink eyes exists in the balanced lethal stock and is usually hidden through enforced heterozygosis. But it comes occasionally to the surface through crossing over. Other recessives might do the same thing. We refer to occasional pure recessives produced by crossing over in balanced lethal stocks as *spurious mutations*.

Muller was first to make up balanced lethal stocks of the kind above described. This he did in order to demonstrate the method of occurrence of the spurious mutations in *Oenothera lamarckiana*.

Balanced Lethals and Translocations in Oenothera. —Balanced lethals occur in a state of nature. The outstanding case in illustration of their natural occurrence is the evening primrose, *Oenothera lamarckiana* (Fig. 134). But in Oenothera one *group* of chromosomes is balanced against another group, not just one chromosome against its homologue, as in the Beaded stock.
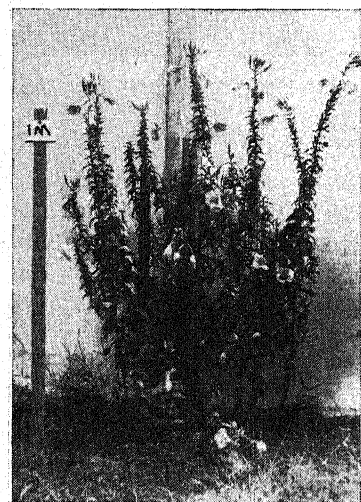
FIG. 134. *Oenothera lamarckiana* (the evening primrose). (From B. M. Davis.)

This grouping of the chromosomes has come about through translocations, as can be seen from the following considerations. Assume that we begin with two normal chromosomes $1 \cdot 2\ \ 3 \cdot 4$,

each segment of the two chromosomes having received a number (Fig. 135a). A translocation is now supposed to result in an interchange of segments 2 and 4, giving the two translocated chromosomes 1·4 3·2. A plant might then be got which is heterozygous for the translocation; that is, one which contains the two translocated chromosomes (1·4 3·2) and the two normal (1·2 3·4). In Drosophila a translocation heterozygote of this composition would form a four-armed figure at the reduction division as shown in Fig. 135b because like segments (as 1 and 1) would pair over
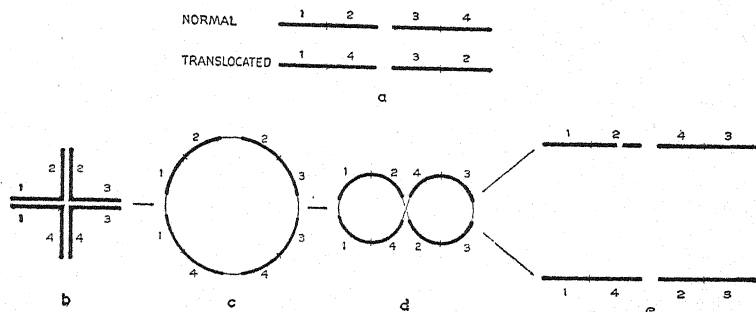
Fig. 135. The grouping of chromosomes in a translocation heterozygote.

their entire length. But in Oenothera they would pair only at their ends, as shown in Fig. 135c. Thus they would form a ring. We can indicate the ring by the formula $\begin{vmatrix} 1\cdot2 - 2\cdot3 \\ 1\cdot4 - 4\cdot3 \end{vmatrix}$. Assume now that the ring always twists into the figure 8 (Fig. 135d). Then the two normal chromosomes (1·2 and 3·4) would always face one pole of the dividing cell and the two translocated (1·4 and 3·2) the other pole. As a result only two classes of gametes would be formed, one class containing 1·2 3·4 and the other 1·4 3·2 (Fig. 135e).

Examination of the ring in the above hybrid will show that 1·2 and 3·4 are *alternate* chromosomes, as are also 1·4 and 3·2. Since alternate chromosomes always go to the same gamete, they act as though they were *tied* to each other. If we refer to two or more chromosomes as a *group*, then each of the two groups under discussion acts as a *unit* at the reduction division. Moreover, the two groups act like a single *pair* of homologous chromosomes, since they always pass to opposite poles at the reduction division.

We can indicate this by the formula $\dfrac{1\cdot 4 \quad 3\cdot 2}{1\cdot 2 \quad 3\cdot 4}$, the horizontal line being continuous in order to show that the chromosomes above the line act like one member of an homologous pair, the two below like the other member. Thus we can represent the translocation heterozygote under consideration in either of two ways. If we are thinking of the ring, the formula is $\left|\begin{array}{c} 1\cdot 2 - 2\cdot 3 \\ \\ 1\cdot 4 - 4\cdot 3 \end{array}\right|$. If we are thinking of the two groups which separate as units at the reduction division, the formula is $\dfrac{1\cdot 4 \quad 3\cdot 2}{1\cdot 2 \quad 3\cdot 4}$. We can refer to these two formulas for the translocation heterozygote as the *ring* and the *group* formula, respectively.

By contrast, a plant pure for the two normal chromosomes is indicated by the formula $\dfrac{1\cdot 2}{1\cdot 2} \ \dfrac{3\cdot 4}{3\cdot 4}$. Here the chromosomes form two pairs in the normal manner, and the horizontal line is broken to indicate that we are dealing with two independent pairs of chromosomes. A plant pure for the two translocated chromosomes would be indicated by the formula $\dfrac{1\cdot 4}{1\cdot 4} \ \dfrac{3\cdot 2}{3\cdot 2}$. The members of both pairs would be perfectly matched and they would pair in the normal manner at the reduction division. Each pair would segregate independently of the other. This again is shown by breaking the horizontal line.

Suppose now that a plant were of composition $\dfrac{1\cdot 2 \quad 3\cdot 4}{1\cdot 4 \quad 3\cdot 2}$ and that each group of chromosomes carried a lethal (l) but at different loci. If we let + signs stand for the normal alleles of each lethal, we can represent this plant by the formula $\dfrac{l\ +}{+\ l}$. The two lethals might be located anywhere within their group as long as they were at different loci. Assume now that there is no crossing over between one group of chromosomes and the other. Then each l remains in its group. As a result, only two classes of gametes are formed; namely, (1) l + and (2) + l. At fertilization sperm cells of class (1) can fertilize eggs of either class (1) or (2), and so can sperm cells of class (2) as shown in Fig. 136. Hence three classes

of fertilized eggs are formed in the ratio of $1 \dfrac{l+}{l+} : 2 \dfrac{l+}{+l} : 1 \dfrac{+l}{+l}$.
Because of the lethals the classes that were pure for one group of
chromosomes or the other would die $\left(\dfrac{l+}{l+} \text{ and } \dfrac{+l}{+l} \right)$ and only the
heterozygotes would live $\left(\dfrac{l+}{+l} \right)$. Thus the two groups of chromosomes would be kept in a state of enforced heterozygosis.
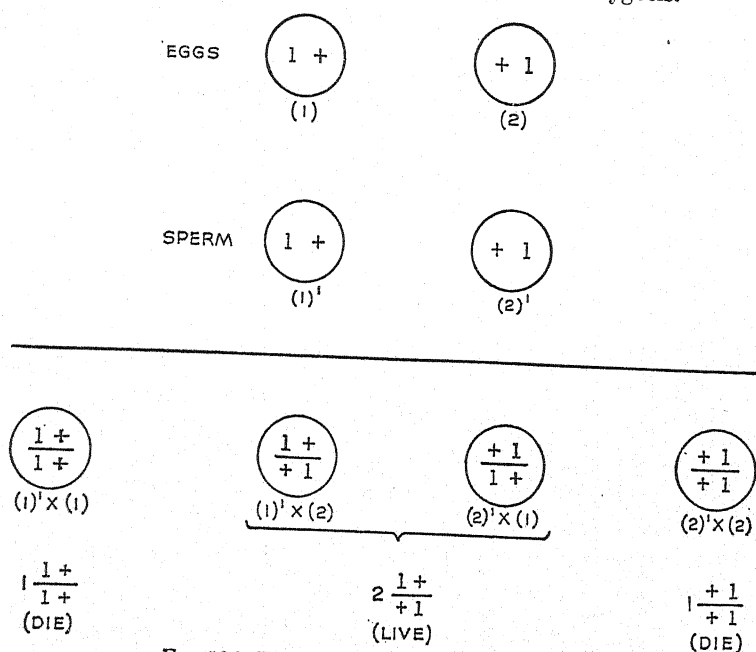
FIG. 136. Enforced heterozygosis in Oenothera.

It will be seen that the translocation has resulted in a ring of
four chromosomes consisting of two groups of two each and that
the balanced lethals then force the plant to remain heterozygous
from one generation to the next for the two groups.

The Increase in Ring-size by Further Translocations.—
Further translocations might increase the size of the ring. Thus
assume that we began originally with three normal chromosomes
and that we numbered them 1·2 3·4 5·6, each chromosome being
divided into two segments and receiving two numbers, one for

each segment. An interchange of segments 2 and 4 would give
1·4 3·2 5·6. Segments 2 and 6 might now become interchanged,
giving 1·4 3·6 5·2. This arrangement in combination with the
normal would give a heterozygote of composition $\dfrac{1\cdot4\ \ 3\cdot6\ \ 5\cdot2}{1\cdot2\ \ 3\cdot4\ \ 5\cdot6}$.
At the reduction division, ends of like segments would come
together (1·4—4·3—3·6, etc.), the chromosomes forming a ring of
six (Fig. 137a). The ring formula for the heterozygote under dis-
cussion is $\begin{vmatrix} 1\cdot4\text{—}4\cdot3\text{—}3\cdot6 \\ 1\cdot2\text{—}2\cdot5\text{—}5\cdot6 \end{vmatrix}$. Assume now that the ring always
twisted into a zig-zag shape, as shown in Fig. 137b. Then chromo-
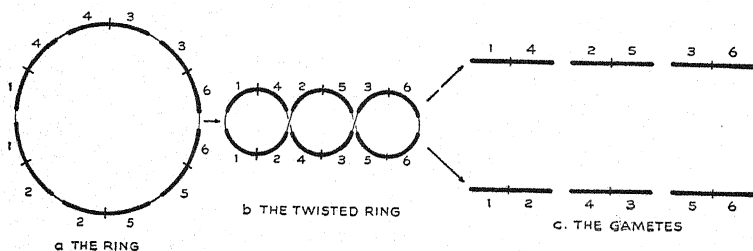
Fig. 137. A translocation heterozygote with a ring of six chromosomes.

somes 1·4 3·6 5·2 would always face one pole of the dividing-
cell, and 1·2 3·4 5·6 the other pole. As a result, only two classes
of gametes would be formed and these would contain the two
original combinations of segments (Fig. 137c). If each combina-
tion contained its own lethal (l), then the only offspring that would
live would be the heterozygous class $\dfrac{l+}{+l}$. Thus the species would
be kept in a state of enforced heterozygosis for the two groups of
chromosomes under discussion.

In the above example it was assumed for the purpose of illus-
tration that the second translocation involved a normal chromo-
some (5·6) and one previously involved in translocation (3·2).
But the second translocation might of course have involved any
two chromosomes. Let us therefore assume that the second trans-
location had involved two chromosomes, neither of which was in
the ring, say, 5·6 and 7·8, and that segments 5 and 8 had become
interchanged, giving us new chromosomes 5·8 7·6. In other words,

the first translocation gave us $1 \cdot 4 \quad 3 \cdot 2$ and the second $5 \cdot 8 \quad 7 \cdot 6$, so that we now have $1 \cdot 4 \quad 3 \cdot 2 \quad 5 \cdot 8 \quad 7 \cdot 6$. Assume also that a gamete with this combination of segments combined with one having the normal arrangement $1 \cdot 2 \quad 3 \cdot 4 \quad 5 \cdot 6 \quad 7 \cdot 8$. Then the heterozygote would be $\dfrac{1 \cdot 4 \quad 3 \cdot 2 \quad 5 \cdot 8 \quad 7 \cdot 6}{1 \cdot 2 \quad 3 \cdot 4 \quad 5 \cdot 6 \quad 7 \cdot 8}$. At the reduction division the ends of like segments would come together, and the ring formula of the heterozygote under discussion would be

$$\begin{array}{c} 1 \cdot 4 {-\!\!} 4 \cdot 3 \quad 5 \cdot 8 {-\!\!} 8 \cdot 7 \\ \Big| \qquad \quad \Big| \; \Big| \qquad \quad \Big| \\ 1 \cdot 2 {-\!\!} 2 \cdot 3 \quad 5 \cdot 6 {-\!\!} 6 \cdot 7 \end{array}$$

. In this heterozygote, *two* rings would be formed, each having four chromosomes, not one ring having six as before.

A new pair of chromosomes is added to a ring only when one member of the pair and one in the ring are involved in translocation. Moreover, it makes no difference which chromosome in the ring is involved: the pair will be added. In the illustration we chose (the one previous to the last) we assumed that the second translocation involved one of the translocated chromosomes in the ring. But it might equally well have involved one of the normal. Thus let us begin again with a pure normal plant and consider just three pairs of chromosomes. This plant would be $\dfrac{1 \cdot 2 \quad 3 \cdot 4 \quad 5 \cdot 6}{1 \cdot 2 \quad 3 \cdot 4 \quad 5 \cdot 6}$. An interchange of segments 2 and 4, say, in the upper group, would give us $\dfrac{1 \cdot 4 \quad 3 \cdot 2 \quad 5 \cdot 6}{1 \cdot 2 \quad 3 \cdot 4 \quad 5 \cdot 6}$. Let us now assume that the second translocation involved one of the normal chromosomes in the ring, say, $3 \cdot 4$, shown below the longer line, and a member of the normal pair $5 \cdot 6$, and say that this translocation consisted of an interchange of segments 4 and 6 (shown below the lines), giving us $\dfrac{1 \cdot 4 \quad 3 \cdot 2 \quad 5 \cdot 6}{1 \cdot 2 \quad 3 \cdot 6 \quad 5 \cdot 4}$. In this translocation heterozygote the ends of like segments would as usual come together at the reduction division and the ring formula would be

$$\begin{array}{c} 1 \cdot 4 {-\!\!} 4 \cdot 5 {-\!\!} 5 \cdot 6 \\ \Big| \qquad \qquad \qquad \Big| \\ 1 \cdot 2 {-\!\!} 2 \cdot 3 {-\!\!} 3 \cdot 6 \end{array}$$

. The ring would as before twist into a zig-zag shape, so that alternate chromosomes in the ring would face the same pole. Chromosomes $1 \cdot 4 \quad 2 \cdot 3 \quad 5 \cdot 6$ would face one pole, $1 \cdot 2 \quad 3 \cdot 6 \quad 5 \cdot 4$ would face the other, and only gametes with these two original combinations of segments would

be formed. If each combination carried its own lethal and there were no crossing over, then the only viable class of offspring would be the heterozygotes, and these would be of the same composition as the parent plant.

In brief then, whenever a translocation takes place between two pairs of chromosomes, the two pairs form a ring; and the ring can be increased in size by further translocations between the ring and new pairs.

The members of the ring in any case fall into two alternating groups of chromosomes, and each group acts as a unit at the reduction division in that all the chromosomes of the group always pass to one pole of the dividing cell. Moreover, there is very little crossing over between the two groups, and each group carries its own lethal. As a result, the species remains in a state of enforced heterozygosis for two *groups* of chromosomes from one generation to the next.

In *Oenothera lamarckiana* translocations have caused six chromosomes to go together in one group and six in another, and the species is kept heterozygous for the two groups by means of balanced lethals. The essential difference then between the Oenothera case and the Beaded case is that in Oenothera one *group* of chromosomes is balanced against another group; in the Beaded stock, just one chromosome is balanced against another (its homologue). The group acts as a unit in Oenothera as a result of translocations. It was suggested independently by Belling and by Darlington that translocations cause several chromosomes to act as a unit in Oenothera. Thus they accounted for the difference between the Beaded and the Oenothera cases.

Spurious Mutations in Oenothera.—Spurious mutations occur in *Oenothera lamarckiana* and they have essentially the same explanation as the spurious mutations artificially produced by Muller in the Beaded stock of Drosophila. Oenothera, we just saw, is normally heterozygous for two groups of chromosomes, each group having its own lethal. If we represent only the lethals and their normal alleles, as we did above, the genotype of the plant is $\frac{l+}{+l}$. But the plant is also heterozygous for red veins and other mutant genes in the two chromosome groups. If we let r stand for red veins and $+$ for its normal allele, then the formula of the

plant becomes $\dfrac{l + r}{+ \, l \, +}$ (assuming that r is in the upper group and its normal allele $+$ in the lower). Red veins are recessive, and therefore the plant shows no evidence of them.

In the absence of crossing over, the Oenothera plant above represented $\left(\dfrac{l + r}{+ \, l \, +}\right)$ forms just two classes of gametes; namely, $l + r$ and $+ \, l \, +$. When these combine they form only one class of offspring that are capable of surviving; namely, $\dfrac{l + r}{+ \, l \, +}$. The recessive gene r again fails to express itself and as the vast majority of the offspring are non-crossovers, the species as a rule gives no evidence of red veins from one generation to the next. But by crossing over in the above hybrid $\left(\dfrac{l + r}{+ \, l \, +}\right)$, r and its allele $(+)$ might exchange positions, giving $\dfrac{l + +}{+ \, l \, \ r}$, and so a gamete might be formed of class $+ \, l \ \ r$ (shown below the line in the last formula). If $+ \, l \ \ r$ combined with an ordinary non-crossover gamete of class $l + r$ (shown above the line in the first formula), it would produce an offspring of genotype $\dfrac{l + r}{+ \, l \ \ r}$. This would be heterozygous for both lethals and would live. But it is pure for r and therefore has red veins.

In effect, the recessive gene (r) has *escaped* from its own lethal by crossing over and the gamete thus formed $(+ \, l \, r)$ gives rise to a viable offspring pure for the recessive gene (r). But this happens only very occasionally, as crossing over is relatively infrequent. One might therefore get the impression that the offspring with the recessive trait (red veins) was a mutant. The exceptional offspring is a *spurious* mutant, not an actual mutant. The particular ratio in which the spurious mutant appears depends upon the amount of crossing over between the recessive gene (r) and its lethal (l). Crossing over is limited to the ends of the chromosomes within a ring, and so only the recessives in the ends can express themselves as spurious mutants. The balanced lethals are located toward the middle of their chromosomes, not the end, and very rarely if ever escape from their respective group.

It will be seen that spurious mutations in Oenothera have the same explanation in principle as the spurious mutations in the Beaded stock of Drosophila. In both cases *the so-called mutant is the result of crossing over in a balanced lethal stock*. But, as previously stated, in Drosophila only two chromosomes are balanced against each other; in Oenothera, two groups.

Synapsis in Oenothera.—In the ancestry of *Oenothera lamarckiana* translocations have resulted in a ring of twelve chromosomes belonging originally to six independent pairs. There is in addition one pair of chromosomes which did not enter into the ring. Figure 138 is a drawing of the chromosomes of *Oenothera lamarckiana* as actually seen at the reduction division.
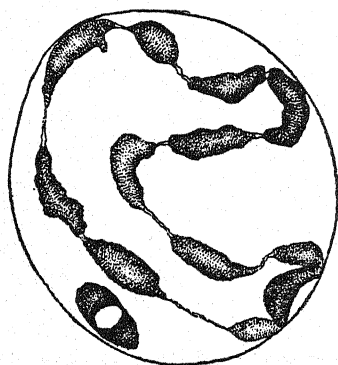
FIG. 138. Synapsis in *Oenothera lamarckiana*. (From Cleland in *Zeit. f. Ind. Abst. u. Ver.*)

It will be recalled that the coming together of chromosomes at the reduction division is known as *synapsis*. In most animals and plants the chromosomes run in pairs of perfectly matched mates, and as corresponding parts pair (gene for gene), the members of each pair come together side by side along their entire length at the reduction division. This method of coming together is known as *para-synapsis* (or side by side pairing). In Oenothera the chromosomes in a ring come together at their ends. This is known as *telo-synapsis* (or end to end pairing). However, in the ring ends of *like* segments always come together. Darlington has suggested that numerous small inversions prevent the pairing of the segments along their non-terminal portions. The segments do, however, pair over a short distance along their ends at the ring stage, and crossing over occurs between the ends. It is obvious that telo-synapsis is just a modified form of para-synapsis.

Chromosome Complexes in Oenothera.—There are a number of different species of Oenothera and most of them are heterozygous for translocations. All of these form rings at the reduction division, the size of the ring depending on the number of chromosome pairs that have been drawn into it by translocations. But

there is one species of Oenothera which does not form a ring and which therefore is not heterozygous for any translocations at all. This is *Oenothera hookeri*. It is probable that *O. hookeri* is the ancestral form from which the remaining species of Oenothera were derived through translocations, balanced lethals, and mutations. In the various species of Oenothera the haploid chromosome number is seven, and in *O. hookeri* we can assign a number to the halves of each of the seven thus: $1 \cdot 2$ $3 \cdot 4$, etc. A translocation between any two or more members of a set produces a set with a new segmental arrangement. A set of chromosomes having a given segmental arrangement is known as a *chromosome complex*.

A species which was hybrid for two complexes would form one or more rings, and the chromosomes within a ring would belong to two groups each with a characteristic segmental arrangement (such as $1 \cdot 2$ $3 \cdot 4$ and $1 \cdot 4$ $2 \cdot 3$). But the two groups within the ring do not in themselves constitute the two complexes if there are chromosomes outside. By definition a complex includes *all* the chromosomes in a set, and in Oenothera a set consists of *seven* chromosomes—the number found in a gamete. If there were just four chromosomes in a ring and the rest in pairs, two of those in the ring would belong to one complex, two to the other. But each complex would include five additional chromosomes, and these would be alike in the two complexes.

In some species of Oenothera all seven pairs of chromosomes are included in a ring. In these cases the two groups that constitute the ring comprise the same chromosomes as the two complexes for which the species is hybrid. In *O. lamarckiana* the ring has incorporated six of the seven chromosome pairs, and in this case the two ring groups and the two complexes are almost the same.

The segmental arrangement found in *O. hookeri* itself constitutes a complex. It can be referred to as the *standard* segmental arrangement, or the standard complex. It contains no lethal, since *O. hookeri* is pure for the standard complex. A species could therefore not be constantly hybrid for it and some other complex derived from it, unless a lethal happened to arise in each complex, and in the part of each complex included within the ring. This sort of thing undoubtedly happened early in the evolution of the Oenotheras, so that at one time in the past a species may have been hybrid for hookeri (the standard complex) and the derived complex. But in time segmental interchanges took place in the

complex with the standard arrangement (hookeri), so that neither complex was now standard. In all present day species hybrid for two complexes, neither complex has the standard arrangement (both are derived complexes). Each complex has received a name. In *Oenothera lamarckiana* one complex has been named *gaudens*, the other *velans*. In another species, *O. biennis*, the two complexes have been named *rubens* and *albicans;* in *O. muricata*, *rigens* and *curvens;* and so on. The total set of mutant genes within a
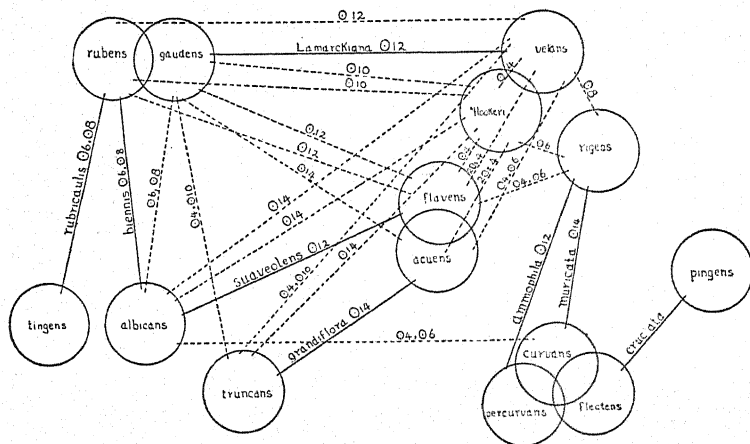
Fig. 139. Ring configurations in *Oenothera* hybrids. (From Cleland in *Amer. Journal of Botany.*)

chromosome complex (both within the ring and outside) is known as a *gene complex*. This has received the same name as the chromosome complex in which it is contained.

It is often possible to cross two species of Oenothera and so to make new combinations of complexes not found in natural species of Oenothera. Thus, *O. lamarckiana* can be crossed to *O. biennis*. This cross will form several different kinds of hybrids including one which contains the two complexes *gaudens* (from *O. lamarckiana*) and *albicans* (from *O. biennis*). In any particular hybrid, whether natural or artificial, the chromosomes form one or more rings, and the number and size of the rings is definite for any given combination of complexes. We can speak of this as the *ring configuration* of the hybrid. In a given hybrid the ring configuration depends on how the segments of one complex are arranged with reference to those of the other complex found in the hybrid. Thus

two complexes, say, *gaudens* and *velans*, would in combination with one another result in a certain ring configuration, but each in combination with some other complex, say, *hookeri*, would form two other ring configurations.

Figure 139 gives the names of various complexes and the size of the rings which they form in combination with one another. Continuous lines connect complexes which are found together in natural species of Oenothera, such as *O. lamarckiana* (combining the *gaudens* and the *velans* complex), *O. biennis* (combining the *rubens* and the *albicans* complex). The broken lines connect complexes which have been brought together by artificial crosses (as *rubens* and *velans*, *gaudens* and *albicans*). To one side of each line is indicated the size of the circle of chromosomes formed in the natural species or in the artificial hybrid which contains the two complexes connected by the line.

Twin Hybrids.—*O. lamarckiana* is hybrid for the two gene complexes *gaudens* and *velans*, and therefore it forms principally two classes of gametes. One contains the complex *gaudens*, the other the complex *velans*. When *O. lamarckiana* is crossed to other species of Oenothera, half the offspring receive *gaudens*, the other half *velans*. Hence two classes of offspring are formed, each showing different mutant traits as determined by the gene complex which it has received. These two classes of offspring are known as *twin hybrids*. Other species of Oenothera also produce twin hybrids in crosses, provided they are hybrid for two gene complexes.

Gamete Lethals.—In some species of Oenothera a lethal kills the pollen cells which contain the lethal. This is known as a pollen lethal. Since the sperm cells are derived from the pollen, the plant cannot form sperm cells containing the pollen lethal. In like manner a lethal might prevent the formation of any egg cells containing it. This is known as an egg lethal. Pollen and egg lethals are referred to as *gamete lethals*.

Suppose now that a plant were hybrid for two complexes, one containing a pollen lethal (l_p), the other an egg lethal (l_e), both within the ring. Then we can indicate the hybrid as $\dfrac{l_p\,+}{+\,l_e}$. This hybrid would form two classes of reduced cells; namely, $l_p\,+$ and $+\,l_e$. But the first class ($l_p\,+$) would not develop into pollen because they contained l_p (the pollen lethal). Thus only $+\,l_e$ pollen would develop and produce sperm cells. In the ovaries only class

l_p + would develop and produce eggs. When the plant was self-fertilized, the only possible combination of gametes would be $+ l_e$ sperm cells and l_p + eggs. Thus all the offspring would be $\dfrac{l_p +}{+ l_e}$. These in turn would produce offspring of the same genotype, and so the species would be in a state of enforced heterozygosis.

The lethals in most species of Oenothera kill the offspring or zygote (as in *O. lamarckiana*) and are known as *zygote* lethals. In these species a plant produces offspring pure for each lethal, but the pures die early in development, leaving only the hybrids. In the case of *gamete* lethals, however, the pures are never formed; only the hybrids. Thus the end result is the same regardless of whether the species is balanced for gamete lethals or zygote lethals.

In some species of Oenothera one of the two balancing lethals is a gamete lethal, the other a zygote lethal. But again the end result is the same as before; only the hybrid offspring live. For let us say a hybrid was of genotype $\dfrac{l_p +}{+ l_z}$, where l_p is a pollen lethal and l_z a zygote lethal. Then only sperm cells of class $+ l_z$ are formed; but egg cells of both classes are formed (l_p + and $+ l_z$), since neither contains an egg lethal. When these are fertilized by $+ l_z$ sperm cells, two classes of zygotes are produced: (1) $\dfrac{l_p +}{+ l_z}$ (lives) and (2) $\dfrac{+ l_z}{+ l_z}$ (dies because it is pure for l_z). Thus again only the hybrids survive.

The Prevalent Ring Size in Various Species of Oenothera. —In the various species of Oenothera, all sorts of chromosome complexes have come together, and these have formed rings of various sizes, from small to large. But the prevalent ring size is large (with 8 to 14 chromosomes in the ring). This is to be expected just as a matter of chance if many translocations have occurred. For Oenothera contains seven chromosomes, divisible into 14 segments, and these 14 segments can be interchanged in a great many ways. Thus very many segmental rearrangements can be formed and not often would any two taken at random be alike. Now the more two complexes differ in their segmental rearrangement, the larger the size of the ring they form in the hybrid. Hence the large ring size found in most species of Oenothera.

The Method of Determining the Segmental Arrangement in the Various Chromosome Complexes.—The chromosomes of Oenothera are not very different from one another in their microscopic appearance, and so it is not possible to determine by direct microscopic examination the segmental arrangement of a given chromosome complex. Nevertheless it is often possible to do this indirectly. For suppose that we begin by assuming no knowledge of the segmental arrangement in any complex. We examine *O. hookeri* under the microscope and see that no chromosomes form rings; all are paired. Hence *O. hookeri* is not heterozygous for any translocations, and we decide to consider it the normal or standard complex. Say, we then combine hookeri and Complex A, and when we examine the hybrid we observe a ring of four chromosomes. These four must represent two from hookeri and two from Complex A, and the two from A must be segmentally rearranged with respect to the two from hookeri (in order to form a ring of four). We now *arbitrarily* number the two from hookeri $1 \cdot 2$ $3 \cdot 4$ and those from Complex A $1 \cdot 4$ $3 \cdot 2$. But these numbers now refer to certain chromosomes, though we might not necessarily be able to identify them under the microscope. The remaining chromosomes would have the same segmental arrangement in hookeri and Complex A, since it is being assumed that they pair in the hybrid. Below, chromosomes with a different segmental arrangement in the two complexes are shown to the left of the vertical line; those with the same arrangement are shown to the right.

Hookeri.........$1 \cdot 2$ $3 \cdot 4$ | $5 \cdot 6$ $7 \cdot 8$ $9 \cdot 10$ $11 \cdot 12$ $13 \cdot 14$

Complex A.......$1 \cdot 4$ $3 \cdot 2$ | $5 \cdot 6$ $7 \cdot 8$ $9 \cdot 10$ $11 \cdot 12$ $13 \cdot 14$

Suppose now that we wanted to determine whether some third complex, call it Complex R, had chromosomes $1 \cdot 2$ $3 \cdot 4$ (of hookeri) or $1 \cdot 4$ $3 \cdot 2$ (of Complex A). Since the chromosomes shown to the right of the line have the same segmental arrangement in hookeri and Complex A (standard in both cases), they form the same number of pairs (whatever this number may happen to be) in combination with Complex R. Now for the two chromosomes to the left of the line. If these are the same in Complex R and hookeri, then they will form two *additional* pairs of chromosomes in the hybrid R-hookeri, so that R-hookeri will have two more pairs than combination R-A. If on the other hand the two chromosomes

to the left of the line are the same in Complex R as in Complex A ($1 \cdot 4$ $3 \cdot 2$) then the combination R-A will form two more pairs than R-hookeri. Thus we could determine whether Complex R had $1 \cdot 2$ $3 \cdot 4$ or $1 \cdot 4$ $3 \cdot 2$. In a similar manner it might be found possible to determine the segmental arrangement of the remaining chromosomes of Complex R (by a comparative study of the chromosome configurations in hybrids formed by combining Complex R with various complexes in which the relative segmental arrangement has been previously determined). The segmental arrangement in a good many complexes has been determined by Cleland by means of comparative studies of the kind just indicated.

Suppose next that we had determined the segmental arrangement of Complex R and that we had done likewise for Complex S by comparative studies of the kind above described. Then we should know what the ring configuration should be in the hybrid R-S (by simply joining the ends of like segments). In all instances in which the segmental arrangement of two complexes has been worked out through comparative studies, the ring configuration in the hybrid formed by combining the two is in accordance with expectation. In other words, the ring configuration of previous untried combinations can be predicted. This therefore is evidence for the correctness of the segmental arrangements in the various complexes as arrived at by comparative studies of the kind above mentioned.

Historical.—The Oenothera case is interesting from an historical point of view. It was upon studies made on *O. lamarckiana* that the Dutch botanist Hugo de Vries based his theory of mutation around the beginning of the present century. Mutations had been known for a long time previous to de Vries' studies, but they had been regarded as freakish events in nature, of no particular significance for evolution. De Vries called attention to their importance, but in a somewhat accidental manner. He observed the spurious mutants of *O. lamarckiana* (along with various abnormal chromosome types) and interpreted them as actually new mutants. This error was quite excusable, for at the time de Vries made his observations, genetics was an undeveloped science and the ground was not yet prepared for the proper interpretation of the Oenothera case.

But spurious mutants do resemble actual mutants in the relative rarity of their appearance, in the suddenness of their appearance,

and in their random character. De Vries said it was changes of *this sort* which were at the basis of evolution. In this respect he was correct, as judged by present day evidence. De Vries might therefore be said to be the father of the mutation theory as applied to evolution. He went too far, however, in considering mutations in general to involve far-reaching changes in characters, resulting in new "elementary species" at one bound. He also thought that mutations were prone to occur at meiosis and that they came in spells or "mutating periods" followed by prolonged periods during which the species underwent no change. These ideas of mutations are not supported by the evidence and are not generally accepted today.

As *O. lamarckiana* came to be studied more intensively by geneticists, certain peculiar features were noticed in its genetic and cytological behavior. For one thing it showed a good deal of sterility, and sterility is a sign of hybridity. (An extreme case of hybrid sterility is that of the mule, the hybrid between horse and donkey.) We now know the meaning of the sterility of *Oenothera lamarckiana*. The offspring homozygous for each complex (*gaudens* or *velans*) are also homozygous for the lethal in that complex and die as young seeds (ovules). The sterility is indeed a sign of hybridity—hybridity enforced through balanced lethals. It was also noticed that the chromosomes of *O. lamarckiana* did not pair side by side (except for one pair) but they seemed to come together end to end. This we saw that they did in the ring. The coming together of chromosomes end to end or telo-synapsis is to be contrasted to the side by side pairing of chromosomes or para-synapsis. Certain cytologists interpreted ring formation in Oenothera as something radically different from para-synapsis and as evidence against the view that chromosomes paired side by side at the reduction division. This we now know is incorrect. In ring formation homologous segments come together, at first part way along their length, and this is essentially para-synapsis.

The various gene complexes were worked out largely by Renner; the microscopic examination of the various hybrids for their ring configuration was done largely by Cleland. It was a very difficult problem, requiring much ingenuity, to work out the combinations of segments and the groups of genes in the various complexes. But the solution of the Oenothera case in terms of our accepted conceptions of genes and chromosomes was fundamentally

dependent upon two theories—the theory of *balanced lethals* and the theory of *translocations*. Balanced lethals account for the fact that the Oenotheras are constant hybrids and that they produce spurious mutants. This was suggested by Muller. Translocations account for the fact that two or more chromosomes act like a single chromosome in the heredity of the Oenotheras. This was suggested by Belling and by Darlington.

Balanced Lethals, Translocations, and Chromosome Complexes in Relation to Evolution.—The Oenothera case is by no means unique in the plant kingdom. An increasing number of species are being discovered in which the chromosomes form rings at synapsis, the result most likely of balanced lethals and translocations.

Of what advantage is this peculiar chromosomal behavior to a species? It will be recalled that hybrids are often more vigorous than the parent races that produce them because of heterosis, and all species that are hybrid for two chromosome complexes benefit by the vigor that comes from hybridity. But in a species with enforced heterozygosis there is always reduced crossing over, and when a species is hybrid for two complexes, each of the two chromosome groups that enter into the ring act as a unit. There is little or no crossing over between them and no independent assortment of entire chromosomes. Thus there are few or no recombinations between mutations belonging to the one group of chromosomes and those belonging to the other.

Now the chief advantage of sexual reproduction, from an evolutionary viewpoint, is that it allows good mutations to be concentrated in a given line through Mendelian recombination. But in Oenothera this would be impossible in the case of mutant genes located in a ring. For if we designate the two chromosome groups in a ring R and S, a plant can never be pure for either R or S. Hence if mutation a arose in group R in one plant and b in group R in another plant (of separate ancestry), it would not be possible to get a and b in the same plant by means of a cross, since a plant containing two R's (pure for R) could not be got (because of the lethal in R). Hence it would not be possible to get a plant pure for a and b through Mendelian recombination. Likewise for mutations of separate origin in group S. It would however be possible to get the two mutations together in the same plant, if the one arose in R and the other in S, but it would not be possible to get

them together in the same complex, provided they occurred in a non-terminal segment of a chromosome ring. Thus it would be impossible to combine into one complex two mutant genes which were located in the ring and which were of separate origin.

The Oenotheras have gained the immediate advantage that comes from heterosis but they have sacrificed the long run advantage that comes from Mendelian recombination in the course of evolution.

SUMMARY

1. Some species breed substantially true even though they are hybrids. The evening primrose (*Oenothera lamarckiana*) is an outstanding example of such a species. But occasionally Oenothera produces aberrant offspring which were originally mistaken for mutations but which actually are recessives and ordinarily are carried by hybrids.

2. In Drosophila it is possible to get flies that are hybrid for Beaded wings (*Bd*), but that breed true. Such flies are of genotype $\dfrac{Bd}{C\ l}$. In this formula, C stands for a crossover suppressor and l for a lethal. (The normal alleles are omitted from the formula but are present in the fly.) Beaded has a recessive lethal effect. Hence flies pure for the Beaded chromosome (*Bd/Bd*) cannot live. Neither can flies pure for the $C\ l$ chromosome $\left(\dfrac{C\ l}{C\ l}\right)$. Hence when two flies of genotype $\dfrac{Bd}{C\ l}$ are bred together, the only offspring that survive are the hybrids $\left(\dfrac{Bd}{C\ l}\right)$.

3. In the above Beaded stock $\left(\dfrac{Bd}{C\ l}\right)$, Bd and l are kept separate by the crossover suppressor (C) and are said to be *balanced* against each other. In general, two lethals that are kept separate by a crossover suppressor are referred to as *balanced lethals*.

4. Balanced lethals keep a stock constantly hybrid, or in a state of *enforced heterozygosis*.

5. It is possible to get Beaded stock of genotype $\dfrac{Bd\quad p}{C\ l}$. In this formula p stands for pink eyes, recessive to red ($+$, not indicated in the formula, but actually present and opposite p). Ordinarily there is no crossing over between l and p, so that flies of the above genotype produce offspring of the same genotype as themselves, for the most part. These show no evidence of p (pink eyes), since p is recessive to red ($+$). But occasionally crossing over takes place between l and p and produces an egg of

class $C\,l\,p$. This, if fertilized by a $Bd\,p$ sperm cell, produces an offspring of genotype $\dfrac{Bd\quad p}{C\quad l\quad p}$. Such offspring are pink-eyed, and since they are rare, they give the outward impression of being mutants. Actually, they are rare crossovers in a balanced lethal stock and they are referred to as "spurious mutations."

6. *Oenothera lamarckiana* is a constant hybrid. It is kept in a state of enforced heterozygosis by balanced lethals in essentially the same manner as is the Beaded stock in Drosophila. But Oenothera is kept hybrid for two *groups* of chromosomes, each of which acts as a unit at the reduction division.

7. The grouping of chromosomes in the Oenotheras comes about through translocations. Thus, suppose $1 \cdot 2\quad 3 \cdot 4$ gave rise to $1 \cdot 4\quad 3 \cdot 2$. Then the translocation heterozygote would be $\dfrac{1 \cdot 4\quad 3 \cdot 2}{1 \cdot 2\quad 3 \cdot 4}$. At the reduction division like chromosome ends come together and form a ring, thus: $\left| \begin{array}{c} 1 \cdot 4\text{---}4 \cdot 3 \\ 1 \cdot 2\text{---}2 \cdot 3 \end{array} \right|$. The ring then twists into an 8 and alternate chromosomes go to the same poles ($1 \cdot 4\quad 3 \cdot 2$ to one pole and $1 \cdot 2\quad 3 \cdot 4$ to the other). Hence only two classes of gametes are formed ($1 \cdot 4\quad 3 \cdot 2$ and $1 \cdot 2\quad 3 \cdot 4$), and in effect $1 \cdot 4$ is linked to $3 \cdot 2$ and $1 \cdot 2$ is linked to $3 \cdot 4$.

8. Further translocations would increase the size of a ring, but in any event the chromosomes in one linked group would alternate with those in the other, and at the reduction division alternate chromosomes would pass to the same pole. Thus only two classes of gametes would be formed, containing one linked group or the other.

9. Each of the above linked groups contains its own lethal. Thus if we let l_1 and l_2 stand for the lethals, then the genotype of any plant is $\dfrac{l_1\ +}{+\ l_2}$. There is very little crossing over between the two groups and therefore each lethal remains in its own linked group. Thus the plant produces only two classes of gametes ($l_1 +$ and $+ l_2$). Therefore when a plant is self-fertilized, it produces offspring in the ratio of $1\ \dfrac{l_1\ +}{l_1\ +} : 2\ \dfrac{l_1\ +}{+\ l_2} : 1\ \dfrac{+\ l_2}{+\ l_2}$. The classes pure for either lethal die, and only the heterozygotes live.

10. In *Oenothera lamarckiana* there are 14 chromosomes. Translocations have resulted in a ring of 12 chromosomes. A single pair remains outside the ring. The chromosomes within the ring form two linked groups of six each, and the species is kept hybrid for the two groups by means of balanced lethals. Each linked group contains recessive genes of its own. In the absence of crossing over the species remains hybrid for the two groups of

genes from one generation to the next and shows no evidence of the recessive genes. But occasionally crossing over takes place and results in a plant pure for a recessive gene. This is a spurious mutation.

11. The number of chromosomes in a ring varies in different species of Oenothera, depending on the number of chromosomes that have become involved in translocations.

12. *In Oenothera hookeri* there is no ring; all the chromosomes are paired. Hence, *O. hookeri* is not heterozygous for any translocations and presumably this species represents the original condition of the chromosomes.

Translocations have caused various segmental rearrangements in the Oenotheras.

13. A chromosome complex is a set of chromosomes with a given segmental arrangement.

14. A ring is formed by all species of Oenothera that are hybrid for two different complexes (or two different segmental arrangements). If the ring does not include all 14 chromosomes, then part of each complex is inside the ring and part outside. A complex always contains seven chromosomes (a haploid set). In *Oenothera lamarckiana* six chromosomes of each complex are inside the ring and one outside.

15. Names have been given to the various chromosome complexes. In *O. lamarckiana* they are called *gaudens* and *velans;* in *O. biennis, albicans* and *rubens;* etc.

16. A gene complex is a group of mutant genes in a given chromosome complex and has the same name as the chromosome complex in which it is contained.

17. In some species of Oenothera, heterozygosis is enforced by zygote lethals; in others by gamete lethals; and in still others by a combination of a zygote and a gamete lethal.

18. It is possible to determine the segmental arrangement of two unknown complexes by combining them with various complexes having known segmental arrangements, and then to predict what kind of a ring configuration should be formed when the two complexes in question are combined.

19. Enforced heterozygosis gives the Oenotheras the increased vigor that usually comes with hybridity, but it prevents Mendelian recombinations and hence deprives the Oenotheras of the long run advantages of sexual reproduction.

PROBLEMS

1. In Drosophila Truncate (*T*, tips of wings cut off) is an autosomal mutation (in the second chromosome) with a dominant visible and a recessive lethal effect (a "yellow mouse" case).

a. Tell why a fly cannot be pure for Truncate.

b. Assume that two Truncate flies are bred together. Give their genotype and tell what kinds of offspring they produce, showing how you derive them.

c. Given a cross of two flies of genotype $\dfrac{T}{C\ l}$ in which T stands for Truncate, C for a crossover suppressor, and l for a lethal, the normal alleles being omitted from the formula (but present in the flies). Tell what classes of fertilized eggs these flies would produce. Tell which classes would not develop and which class would, giving the reason in each case.

d. If a stock of flies was of genotype $\dfrac{T}{C\ l}$, what would be the appearance of the offspring from one generation to the next? Why?

e. In general, what kind of a stock is one which breeds true, though heterozygous? Briefly explain why the suppression of crossing over is necessary if such a stock is to breed true.

2. Given a cross of two flies each of genotype $\dfrac{T}{C\ l\ b}$, T, C and l standing as before for Truncate, a crossover suppressor, and a lethal, and b for black body, recessive to its normal allele (normal alleles again being omitted from the formula, though present in the flies).

a. In the absence of crossing over, what will be the genotype and the appearance of all the offspring? Tell in particular why there will be no black offspring.

b. Assume that in a female of genotype $\dfrac{T}{C\ l\ b}$ there is a slight amount of crossing over between l and b, say, 1 per cent, but that otherwise there is no crossing over (that is, none between T and l, and none at all in the male, there being no crossing over in the male of Drosophila). Then tell whether or not a black offspring might be produced, and if so, show how.

c. Ordinarily if two flies were hybrid for black but did not contain balanced lethals, what proportion of their offspring would be black? Does the balanced lethal stock produce black offspring in this proportion?

d. For what might the occasional black offspring thrown by the above balanced lethal stock be mistaken, if we did not know the genetic constitution of the stock?

3. In *Oenothera lamarckiana* what causes each of two groups of chromosomes to be transmitted as a unit to the offspring? What causes the species to remain hybrid for the two groups from one generation to the next? Tell then how Oenothera and 100 per cent Truncate stock (1) agree in principle and (2) differ.

4. Given a translocation heterozygote of composition $\dfrac{1\cdot4\ \ 3\cdot6\ \ 5\cdot2}{1\cdot2\ \ 3\cdot4\ \ 5\cdot6}$. Make a diagram to show what sort of a figure the chromosomes would

form at the reduction division (1) if homologous segments came together along their entire length (the chromosomes forming a six-armed figure), and (2) if only the ends of homologous segments came together (the chromosomes forming a ring). Might figure (1) give rise to figure (2)? If so, how? Does figure (1) occur in Oenothera?

5. Given five chromosome complexes in Oenothera having the following segmental arrangements (the first having the normal segmental arrangement, or that found in *O. hookeri*).

    a. 1·2 3·4 5·6 7·8   9·10 11·12 13·14
    b. 1·4 3·2 5·6 7·8   9·10 11·12 13·14
    c. 1·2 3·6 5·4 7·8   9·10 11·12 13·14
    d. 1·6 3·2 5·4 7·8   9·10 11·12 13·14
    e. 1·6 3·2 5·4 7·10 9·8 11·12 13·14

Give the ring formulas (or ring configurations) in hybrids produced by the following combinations of complexes and give also the number of chromosome pairs not included inside a ring: (1) $a \times b$, (2) $a \times c$, (3) $a \times d$, (4) $b \times c$, (5) $a \times e$.

6. *Oenothera lamarckiana* has a ring of 12 chromosomes (and one pair outside the ring). What is the minimum number of translocations necessary to produce a ring of twelve chromosomes?

7. Given the four following segmental arrangements.

    a. 1·2 3·4 5·6
    b. 1·4 3·2 5·6
    c. 1·2 3·6 5·4
    d. Unknown.

When these segmental arrangements are combined, they produce the ring configurations listed below.

    $a \times d$—ring of four chromosomes
    $b \times d$—ring of six chromosomes
    $c \times d$—ring of six chromosomes

Tell which chromosomes are segmentally rearranged (or translocated) in *d*. (Hint: Since $a \times d$ gives a ring of four, how many chromosomes in *d* are translocated with respect to *a*? Since $b \times d$ and $c \times d$ both give six, which two normal chromosomes (in *a*) must be translocated in *d*?)

19. *HEREDITY AND DEVELOPMENT*

GENES cause us to resemble our parents because they control development. It is therefore obvious that heredity and development are closely connected, and a consideration of the one leads to the other.

The Theory of Epigenesis.—All the cells of a developing embryo contain the same kinds of genes, yet some cells develop into one kind of organ, others into another kind. Why should they develop differently? In answering this question it should first of all be borne in mind that the genes do not cause a cell to develop into anything in particular but that they give the cell the *capacity* to develop in any one of a great many different directions. The particular direction in which the cell develops is then determined by the environment. Now the cells of an embryo differ in position. Accordingly their environment differs, and this causes them to develop in different directions. In other words, the environment causes *differentiation*.

Often differentiation gets started before fertilization and is due to the position of the egg in the ovary. In the case of the frog one pole of the egg is nearer the arterial blood supply and the opposite pole nearer the venous supply. The first comes to contain less yolk and more protoplasm (the animal pole) and the second more yolk and less protoplasm (the vegetative pole). The animal pole develops into the head end, the vegetative pole into the tail end. But the very early frog's egg has neither animal nor vegetative pole; it is undifferentiated. After differentiation has gotten started, it may be the cause of further differentiation. Often one part of the embryo produces chemical substances which reach other parts and cause them to develop in a definite direction. In this case the one part serves as the environment for the other parts.

We might summarize by saying that the developmental capaci-

ties of a cell are determined by its genes, but the particular direction in which a cell develops is determined by its environment. This is known as the theory of epigenesis.

Chemo-differentiation and the Mosaic Stage in Development.—Differentiation at first involves no visible change in cells but merely a chemical change in their cytoplasm. This is known as *chemo-differentiation.*

In the case of the frog's egg the animal and vegetative poles are visibly different, but this difference is a secondary result of an earlier chemical differentiation, itself invisible. The establishment of the polar axis (the axis from head to tail) is the first step in the chemo-differentiation of the frog's egg. The second step is the establishment of the dorso-ventral axis, as a result of which the embryo becomes divided into left and right halves. In the case of the frog's egg the dorsal surface develops diametrically opposite the point of entry of the sperm cell. Here again, the environment (in the form of the sperm cell) has been the cause of chemo-differentiation.

Further examples of chemo-differentiation might be taken from the newt (a tailed amphibian). In the developing newt it is possible to remove the rudiment of an organ from its normal position, say the limb bud, and transplant it to some other part of the embryo, the tail for example. If this is done at a very early stage in development, the limb bud cells will develop into part of the tail. But if it is done somewhat later, the limb bud will develop into a limb—a limb attached to a tail. Yet the limb bud cells look the same at stage two as at stage one. They are still *visibly* undifferentiated. But at stage two they are *chemically* different from other cells of the embryo and therefore they develop into a limb when transplanted.

Once a cell has become chemically differentiated it tends to complete its differentiation and become visibly differentiated, even in some abnormal position. In general, then, the cells of the newt embryo are at first *plastic* as regards their powers of development (capable of growing into any part when transplanted). Later chemo-differentiation sets in, and from now on the cells pursue definite paths in development more or less regardless of their position in the embryo.

Chemo-differentiation sets in gradually. At first the limb bud cells can grow into anything—tail, liver, eye. A little later they

can develop only into a limb. But for a while any small fragment of the limb bud, say a finger joint, can grow into an entire limb if transplanted. Later lower arm becomes chemically differentiated from upper, and at this stage the finger joint can grow into lower arm but not into the upper. Still later the hand becomes differentiated from the rest of the lower arm, and now the finger joint cannot give rise to any part of the arm above the hand. But all of the hand cells are still alike, and the finger joint can grow into an entire hand. Still later the various parts of the hand become chemo-differentiated and the finger joint can give rise only to finger joint.

After chemo-differentiation has become complete the embryo is divided up into a great many small patches of tissue and each patch develops into some definite organ or some definite part of an organ. The embryo now is in the *mosaic* stage of development. Yet all the cells still look very much alike. They are *invisibly* differentiated; the embryo is a *chemical* mosaic.

To summarize, before chemo-differentiation the embryo is in the plastic stage of development; after chemo-differentiation, it is in the mosaic stage. But the one stage passes gradually into the other. The mosaic stage begins with the first act of chemo-differentiation, the one that results in the long axis; it ends when chemo-differentiation is complete. Moreover, the different parts of the embryo never really develop in complete independence of one another. If for example the optic cup is removed from its normal position, it will not give rise to an eye of typical shape. The various parts of the embryo interact at all stages of development, even in the fully developed mosaic stage.

If some cells are removed from a newt embryo very early in the plastic stage of development, the embryo develops quite normally. Even comparatively large chunks of tissue may be removed and the embryo will develop into a whole embryo, with no missing parts. All that happens is that the cells that are removed are replaced by the surrounding cells. But if a part of the embryo is removed in the mosaic stage, a corresponding part will be missing in the adult. If an entire limb bud is removed, the adult lacks a limb; or, if the hand region of the bud is removed, the adult lacks a hand. Losses at the mosaic stage are irreparable.

The change from the plastic to the mosaic condition takes place in large measure at a definite stage in development. While the

newt egg is still a ball of cells, a lip-like growth appears on the dorsal surface of the embryo. This is known as the *dorsal lip*. Much of the change from the plastic to the mosaic stage takes place at the time that the dorsal lip makes its appearance, and is due to a chemical substance produced by the dorsal lip. However, the mosaic stage really begins with the differentiation of the long axis, and this occurs before the dorsal lip is developed.

The Stage of Reintegration.—The embryo does not remain indefinitely a patchwork of more or less independently developing parts. As development proceeds the blood system and the nervous system develop, and these serve to co-ordinate development in the different parts of the embryo. The blood system conveys hormones from one part of the embryo to another part, and so one part can influence another, as when hormones from the ovaries and testes reach other parts of the body through the blood and cause the secondary sexual characters to develop. The nervous system conducts impulses to various organs, especially the muscles, and nervous impulses sometimes stimulate development by stimulating the activity of an organ. Also, the stress and strains set up by *use* tend to co-ordinate development, as shown especially by bones, in which the laminations and the internal struts develop where the stresses determine. The development of an organ through use is sometimes referred to as *functional differentiation*.

We can, then, roughly divide the development of the embryo into three stages on the basis of the relative independence of parts: first, the *plastic stage*, then the *mosaic stage*, and finally the *stage of reintegration*. The newt embryo can replace parts that are lost in the first and third stages of development. It is only when parts are lost in the mosaic stage of development that the embryo cannot replace them.

Determinate and Indeterminate Eggs.—In some species, chemo-differentiation is well under way before fertilization, and the eggs of these species are said to be of the *mosaic*, or *determinate*, type. The eggs of other species are not chemo-differentiated before fertilization, or only slightly so, and they are referred to as *indeterminate* eggs (because differentiation is not yet determined).

The sea urchin egg is indeterminate. When it divides into two, it is still undifferentiated, and the first two cells, if separated, are therefore capable of giving rise to two complete embryos. The snail's egg, by contrast, is chemo-differentiated (determinate), and

the first two cells are not capable of giving rise to complete embryos. Instead, they tend to develop into half embryos (the two halves of the body having already been determined in the fertilized egg, as the result of chemo-differentiation).

Organizers: Inducers of Development.—Chemical substances (and possibly other agents) may emanate from one part of an embryo and cause differentiation in a neighboring part. This is known as *induction*.

The development of the eye involves a case of induction that has been known for a long time. The eye begins as an outgrowth of the brain on each side of the head (see Fig. 140). This outgrowth eventually forms a cup-shaped structure (the optic cup) attached to the brain by means of a stalk (the optic stalk). The optic cup approaches the surface layer of the embryo (the ectoderm) and there induces the development of a lens. It is possible to remove the optic cup of a newt or frog embryo and transplant it elsewhere below the skin of the embryo. Wherever it is transplanted a lens will develop in the ectoderm cells adjacent to the optic cup. This shows that some sort of chemical or physical agent is emanating from the optic cup and starting off lens formation in the adjacent ectoderm. Parts of the embryo which induce differentiation in neighboring parts are sometimes called *organizers*. The optic cup, then, is an example of an organizer.

FIG. 140. The optic cup—an organizer.

A very striking case of induction has more recently been worked out by Spemann. In the newt embryo the dorsal lip folds in below the surface of the embryo and it induces in the overlying ectoderm the formation of "neural folds," from which nerve cord and brain develop. It is possible to remove the dorsal lip from its normal position and transplant it somewhere else below the surface of the embryo. The dorsal lip will then induce the formation of neural folds in the ectodermal cells above it, even though these particular ectodermal cells normally do not form a nerve cord and brain.

The dorsal lip owes its power of induction to a chemical substance

(a sterol) which diffuses from it to the overlying ectoderm. The substance in question is not specific; that is to say, the substance produced by the dorsal lip of one species, say the newt, is not peculiar to that species. The frog forms the same substance, so that the dorsal lip of the frog can induce neural folds in the newt, and vice versa. In fact, it is probable that other tissues of the embryo produce the same substance, though not so much of it. It is produced even by cancer cells and by widely distinct forms of life (the primitive streak of chicks and by coelenterates). The substance in question is somewhat comparable to a hormone, the main difference between the two being that the one is formed early in development before there is a blood system to transport it, the other (the hormone) later when there is a blood system.

The dorsal lip itself contains the rudiments of the notochord (the precursor of the backbone). It also contains plates of tissue known as the mesoderm plates, which give rise to the muscles, kidneys, blood vessels, and in fact almost all the tissues and organs of the body included between the surface layer (the ectoderm) and the lining of the gut (the endoderm). Thus the dorsal lip not only induces the formation of a nerve cord and brain in the ectoderm, but it also contains the rudiments of many other organs of the newt's body. Hence when the dorsal lip is transplanted to some other part of the embryo, not only do the spinal cord and brain start to develop there but in addition all the organs of the body that arise from notochord and mesoderm plates. The dorsal lip therefore appears to control almost the whole of development, and it has been called *the* organizer. However, the principal effect of the dorsal lip *in its capacity as an organizer* is the induction of neural folds in the overlying ectoderm. Now, the dorsal lip could not induce neural folds unless the ectoderm had the capacity to form them. This capacity is dependent upon genes. Even the dorsal lip itself owes its capacity for induction to its genes.

Fields.—A chemo-differentiated region of the developing embryo is sometimes referred to as a *field*. In every field developmental activity is highest at some one point, and it becomes gradually less the further we go from this point. In other words, the field consists of an *activity gradient*. The first established and the main field of the newt embryo is its long axis (from head to tail), and within this field the head end is the region of highest activity. The second field to be established is the dorso-ventral field with

high point in the dorsal lip. Another important field is one which determines the asymmetrical disposition of the heart and viscera from left to right sides.

The activities within any field of development are of a highly complex nature and involve all sorts of physical and chemical changes. Among the chemical changes oxidations are very common, and within any field there is a gradient of oxidation corresponding to the gradient of general activity in the field. Now oxidations are among the most easily detectable of chemical changes, and it was through them that the main axial gradient (from head to tail) was discovered. This discovery was made by Child, and it was at first thought that the gradient of oxidation determined differentiation. It is now thought that differentiation is the result of the general activities within a field, and that oxidations are merely one expression of these general activities rather than the cause of them. The cause itself ultimately comes down to the genes within the egg and the environment within which the genes act.

Since a field always has a gradient, and a gradient is always indicative of a field, the two are inseparable and we can refer to them jointly as a *gradient-field*.

Fields in Relation to Regeneration and Regulation.— Suppose we should cut an earthworm into a number of pieces, each a section of the long axis. Then each piece would grow a new head at its fore end and a new tail at its hind end. Accordingly, the earthworm is said to have *polarity*. This polarity is due to the fact that in the adult earthworm the main axial gradient still exists, and when the animal is cut into a number of pieces, the fore end of each piece is at a higher point in the gradient than the hind end. Accordingly, a head develops at the fore end of each piece. Once the head gets started, it causes the hind end to develop into a tail. Within any field the high point dominates the lower points and causes them to develop into proper relation to the high point and to one another. In the case of the main axial field, the head end is the high point and it dominates the rest of the field.

Suppose next that we should cut an earthworm transversely into two, as shown in Fig. 141, left. Then the cells just behind the cut (represented by the shaded band) would be at the front end of the hind piece and accordingly they would develop into a head. We might, however, have made the cut just behind the layer of cells under discussion, as shown in Fig. 141, right. In this case they

would have been at the hind end of the front half and would have developed into a tail. Thus one and the same layer of cells might develop into either a head or a tail, and this would depend on the level of the layer in the gradient-field of the piece to which it belonged. The *entire* field therefore determines what one of its parts grows into. It acts as a *unit* in development; it is not a mosaic, in which each part acts independently of the rest. This applies to fields in general, whether in the embryo or the adult.

When an earthworm is cut transversely into pieces, each piece of course contains just a section of the axial gradient. But after the head and tail have grown back, the normal axial gradient is restored. In the adult, lost parts are usually restored by means of growth and cell division. This is *regeneration*. But in the very early embryo, lost parts are at first restored without cell division and growth. The parts that remain become remolded to form a complete but smaller embryo.

HEAD END

BECOMES A HEAD ⟶ ⟵ BECOMES A TAIL

TAIL END

FIG. 141. The control of a part by its field in regeneration (in the earthworm).

This is known as *regulation*. If in the early embryo a field were divided into halves, each half would at first lack part of the normal gradient, but by a process of regulation the entire gradient would be restored.

We can think of the entire gradient within a field as representing a state of *equilibrium*. A field tends to return to a state of equilibrium whenever its gradient is altered. In the adult a damaged field tends to restore its equilibrium through cell division and growth. This constitutes regeneration. In the early embryo the field restores itself by remolding the tissues at hand. This constitutes regulation.

Twinning.—If we should get a developing frog's egg at the two-cell stage, we might separate the first two cells by tightly tying a string between them. Normally each of the first two cells would give rise to one-half of the adult frog. But when they are

separated each cell restores the missing half and gives rise to an entire frog. Thus identical twins are produced. If the first two cells had been separated only part way, then they would have given rise to Siamese twins.

Identical twins occur naturally in man and other forms of life, but when twinning occurs naturally the division of the embryo

Fig. 142. Coalescent twins. (From an article by Helen Black in *The Journal of Heredity*, based on articles by Professor Anukhin of Moscow.)

into two takes place later than the two-cell stage. It is not known just what causes the embryo to divide into two separate embryos, but the cause is thought to involve some interference with the main axial gradient. Anything which depresses the axial gradient at the head end might result in two secondary high points somewhat further back and on each side of the mid-line. This would then cause two heads to develop, followed by the division of the embryo down the mid-line. When the division is incomplete, the result is Siamese twinning (Fig. 142).

In the Texas Armadillo the young are always born in a litter consisting of four identical twins (or rather quadruplets). They arise from a mass of cells which branches into four parts, each

part developing into a separate embryo. It has been observed that development is arrested for a period of about three weeks preceding the stage when the embryo branches into four parts. The arrest in development seems to be due to a delay in the implantation of the embryo in the uterine wall of the mother. As a result the embryo does not receive its usual supply of oxygen. The head end of the embryo is ordinarily more active in development than the regions farther back, and accordingly its oxygen requirements are higher. For the same reason it is more sensitive to a lowered oxygen supply. It has been suggested that the relative lack of oxygen attendant upon delayed implantation of the embryo causes the division of the head region and that this is followed by the division of the rest of the embryo.

The production of identical twins has an hereditary basis in the Texas Armadillo, since it occurs normally (that is, apart from special environmental agents). It probably also has an hereditary basis in man and is dependent on a mutation, perhaps one which somehow depresses the axial gradient at the head end of the embryo.

Hormones in Man and Other Vertebrates.—It will be recalled that in man and other vertebrates the ovaries and testes produce chemical substances, referred to as hormones, which get into the blood and influence the development of the secondary sexual traits. The ovaries and testes are referred to as glands of internal secretion or *endocrine glands* from the fact that they throw their secretion into the blood. There are several other endocrine glands in the body besides the ovaries and testes and each secretes its characteristic hormone or hormones. The various hormones have a large variety of effects on the body, but for the present we are interested only in those which influence development.

The *thyroids* are glands located in front of the windpipe. These glands produce a hormone known as *thyroxin* which increases the rate of all oxidations in the body and so it whips up all activities dependent on oxidations. Thyroxin is a complicated chemical substance containing the usual chemical elements found in the body plus an element not so usual, namely, *iodine*. Thyroxin owes its unusual properties to iodine.

Derangement of the thyroid has different effects, depending on whether the derangement occurs in the adult or in the growing

individual and whether it involves overactivity or under-activity of the gland. If the thyroid is underactive in a child, the oxidations necessary for normal growth do not take place at the proper pace, and the child becomes a *cretin*. Thyroid administration often cures cretinism.

In frogs the thyroids are necessary for the metamorphosis of the tadpole into an adult. Tadpoles from which the thyroids have been removed remain tadpoles indefinitely. On the other hand, if very young tadpoles are fed thyroid gland (or if given thyroxin), they metamorphose within a few days and give rise to frogs of adult form but very diminutive in size (Fig. 143). Iodine can also cause metamorphosis, but it is slower-acting than thyroxin because iodine does not act directly; it must first be combined with other atoms to form thyroxin before it can act.
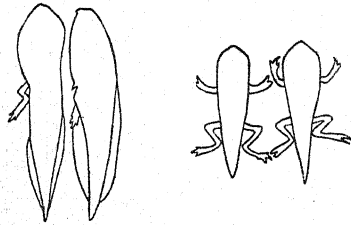
FIG. 143. The effect of thyroxin on tadpole metamorphosis. Controls on the left. On right, tadpoles of the same age after seven days' exposure of 1/1,000,000 synthetic thyroxin. (From Harrington, *The Thyroid Gland*, Oxford University Press.)

In frogs of ordinary size the thyroid releases its thyroxin after the tadpole has undergone about three months' development, but in bull frogs the thyroid does this only after the tadpole has been developing for two years and has grown correspondingly large. In the newt known as the Mexican axolotl the thyroid never releases its thyroxin and the newt ordinarily remains indefinitely in the tadpole stage. But if the axolotl is fed some thyroid (or thyroxin) it metamorphoses and becomes a newt of adult form.

Newts and salamanders, though rather close relatives of the frog, cannot be made to lose their tails by thyroid administration. This shows that their tails are less sensitive to thyroxin than are the tails of frog tadpoles. Thus metamorphosis depends on two things (1) thyroxin and (2) the sensitivity of the cells to thyroxin. We are dealing here with a situation very similar to that found earlier in development, where the development of the neural folds depends on (1) a chemical substance produced by the organizer (the dorsal lip) and (2) the sensitivity of the ectodermal cells to the chemical substance, as expressed by the development of neural folds.

It is not necessary to use human thyroid to cure cretinism in man; sheep's thyroid or any other thyroid will do. Moreover, sheep's thyroid (or any other kind) can be used to induce metamorphosis in tadpoles. This shows that the thyroxin of different animals is substantially the same. Thyroxin must have originated very early in evolution, at the time the vertebrates were in the fish stage of evolution, since the lower and higher vertebrates have thyroxin in common.

Another gland of importance in development is the *pituitary*. This is a small gland about the size of a pea located between the base of the brain and the roof of the mouth, referred to on a previous occasion (p. 128, Fig. 50). The pituitary, though very small, produces a large number of hormones. Most of these hormones have no effect in their own name, so to speak, but they stimulate other glands into activity and so cause them to produce their hormones. The pituitary, through its hormone or hormones, is necessary for the proper development of the ovaries and testes; it also stimulates the production of oestrone and

FIG. 144. Infantilism. Comparison of a case at age of 9 yrs. 6 mos. with a normal boy of the same age. (From Engelbach in *Endocrinology*.)

androsterone by the ovaries and testes in the adult. It will be recalled that if the pituitary is underactive before the age of puberty, the reproductive organs fail to develop normally and a derangement develops known as Fröhlich's syndrome. The pituitary also produces a hormone necessary for the growth of the entire body. If the pituitary is underactive from a period beginning before birth, the body remains small and childlike in proportions. This derangement is known as *infantilism* (Fig. 144). On the other hand, if the pituitary is overactive very early in life, it causes *gigantism* (Fig. 145). Overactivity of the pituitary later in life causes *acromegaly*. This derangement is characterized by enlargement of the bones of the head, hands, and feet; that is, the bones at the ends of the body.

Then again there is a certain pituitary hormone which is necessary for the utilization of fat by the body. If this is deficient, fat accumulates in the body and the result is pituitary obesity.
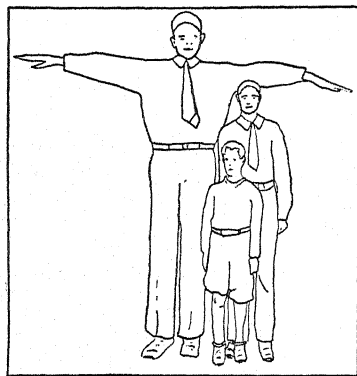
Fig. 145. Gigantism. The patient at the age of 13 is shown with his brother who is 9, and his father. (From Behrens and Barr in *Endocrinology*.)

The pituitary stimulates the thyroid both in man and the frog. If the pituitary is removed in a tadpole the thyroid fails to develop and the tadpole does not metamorphose.

Insect Hormones.—A hormone can be regarded as a trait, and it is like any other trait in that its development depends on genes. This has been demonstrated in the case of insects. As an example, we might give the experiments of Caspari on the flour moth, *Ephestia kühniella*.

In the flour moth the eyes are normally black but there is a red-eyed mutant (a recessive) which lacks the dark eye pigment. It is possible to transplant the testis of a black-eyed larva into a red-eyed larva and when this is done the red-eyed larva develops black eyes. The ovary or brain of the black-eyed race might equally well be used. This shows that the testis, ovary, and brain normally produce some hormone necessary for the development of black eyes. It is possible to extract the hormone (by means of alcohol or acetone) and to make the larva of the red-eyed race develop black eyes by injecting the extract into it. The hormone is effective in extremely minute amounts. For when normal tissue, say testis, is transplanted into a red-eyed female when a larva, a minute amount of the hormone gets into the eggs of the female, and this is sufficient to make her offspring develop black eyes. But the effect of the hormone disappears in the next following generation.

These experiments show not only that eye development in the flour moth depends on a hormone but they also show that the hormone in turn depends on genes. For the hormone fails to develop in the red-eyed race because a certain normal gene is lacking in the red race. The hormone is therefore under gene

control, and this is the point to be emphasized in the present connection.

Hormones have also been found to be under gene control in Drosophila, in experiments of Ephrussi and Beadle. The rudiments of the eyes are present in the larva of Drosophila in the form of disks. An eye disk can be removed from one larva and transplanted into the body cavity of another. Here it grows more or less as it would have in its normal position and gives rise to an eye. In general, if the eye disk of a mutant race is transplanted into a normal larva, it develops into the mutant-type eye. Development is said to be *autonomous* (independent of the host). These cases afford no evidence of hormone action. But there are two exceptional cases involving the eye disks of two mutant races known as vermilion and cinnabar (both with eyes lighter than the normal red). If the eye disk from a larva of either of these races is transplanted to a normal larva, it develops into a normal red eye. The normal larva must be supplying one or more hormones which make the mutant eye disk develop into a normal red eye. The experiments indicate that two hormones are involved (call them A and B). Vermilion lacks both A and B but cinnabar lacks only B. Normally A is formed first and then B. Moreover, B cannot be formed if A is lacking, and vermilion lacks both A and B because it cannot produce A. It can, however, produce B if A is supplied. When a vermilion eye disk is transplanted into a cinnabar host, it is supplied with A by its host. The vermilion disk can now make B and it then develops into a normal red eye. But a cinnabar disk in a vermilion host develops into a cinnabar eye, because cinnabar needs B to develop into red, and the host tissue (vermilion) does not supply B. (It is true perhaps that a small amount of A diffuses out of the cinnabar eye into the vermilion host but this is not sufficient to cause the vermilion host to form B.) Here as in the previous case the important point is that the hormones are known to be under gene control, since they cannot be produced in the mutant races but can in the normal.

The hormones concerned with eye-color development are not specific for a given species; that is to say a certain kind of hormone is not limited to that species. Extracts of the hormone produced by the testis, ovary, and brain of Ephestia, previously referred to, can cause the disk of a vermilion Drosophila to develop into a normal red eye. This shows that the hormone of Ephestia can

act as a substitute for the hormone which vermilion cannot produce (*A*) and so the two hormones are probably not very different. Now moths and flies are very different orders of insects, and it is rather remarkable that they should have a hormone in common. The hormone must have arisen very early in evolution, at a time when the different orders of insects had a common ancestor, and both moths and flies then inherited the hormone from their common ancestor. The chemical substances in the living world apparently are of great antiquity.

The Influence of Genes on the Rate of Development.— Genes often produce their effect by changing the rate of development. This is well illustrated by experiments of Ford and Huxley on the small shrimp-like animal Gammarus. The eyes of Gammarus are normally black, but there are various mutant races with lighter eye colors, including red, brown, and dark brown. The eyes of the normal race are at first red, but they get darker very rapidly by the formation of black pigment and are black before the embryo is fully developed. In the dark-brown race, black pigment is laid down more slowly than in the normal black race, and the eye color does not develop beyond the dark-brown stage before the shrimp is an adult and pigment formation ceases. In the lighter-brown race the dark pigment is laid down still more slowly and the eye color does not get beyond the lighter-brown stage. In the red race no dark pigment is formed, possibly because pigment formation is slowed down to zero amount.

The Gammarus case is not unusual; it has been found that genes often produce their effects by influencing the rate of development.

The Problem of Cytoplasmic Inheritance.— Some embryologists are of the opinion that the cytoplasm determines important traits and that the genes in the chromosomes are concerned with only minor varietal differences between individuals within a species. This view is based partly on crosses between animals of widely different species. For example, it is possible to fertilize the eggs of a sea urchin with sperm of a mollusc. The fertilized egg does not develop into an adult, but it does develop into an early larva, and it becomes the larva of a sea urchin, not a mollusc. This has been interpreted to mean that the egg cytoplasm determines development in the main and that the sperm nucleus was without influence. It is possible, however, that in the present case the sperm nucleus merely activated the egg to development

and that it degenerated soon after fertilization or failed to function because of the foreign cytoplasm. In this event, the egg would in effect have developed parthenogenetically.

It is possible actually to fertilize sea urchin eggs of one species with sperm of another, and when this is done, the larva definitely develops paternal, as well as maternal, traits. However, the very early larva often shows predominantly maternal traits, and only later does it show the paternal influence. This is undoubtedly due to the fact that the maternal nucleus has impressed its character on the egg cytoplasm before fertilization, and it then takes time for the sperm nucleus to exert its modifying influence on the cytoplasm.

The Delayed Effect of Genes.—We do in fact know that the egg cytoplasm might be influenced by the genes of the mother before fertilization. This has been demonstrated in the fresh water snail (Limnaea) by Boycott, Diver, and Garstang. The shell of a snail normally turns to the right (is dextral) but there is a recessive mutant with a left-turning (or sinistral) shell. We can designate the mutant gene as *s* (sinistral) and its normal allele as $+$. The coiling of the shell can be traced very far back in development, and the difference between a dextral and sinistral snail can be seen as early as the second "cleavage" (or cell division) of the fertilized egg. In fact, the difference is potentially present in the *unfertilized* egg. Now the direction in which the developing egg coils is not determined by its own genes but by the genes of its mother. The egg, of course, is derived from an unreduced cell (the diploid oöcyte), and this has the same genotype as any other unreduced cell of the mother. Suppose then that the mother were left turning, and of genotype s/s. This formula would apply to all her unreduced cells, including her oöcytes (Fig. 146). In these oöcytes the cytoplasm would become left-turning due to the s/s constitution of their nucleus. The eggs derived from the oöcytes would therefore develop into left-turning snails, even though they were fertilized by $+$ sperm (containing the dominant normal allele for dextral, Fig. 146). But in the hybrid the unreduced eggs (oöcytes) would have a nucleus of constitution $+/s$, and since $+$ (dextral) is dominant, the cytoplasm of these unreduced eggs would become dextral. Hence all the *reduced* eggs would have dextral cytoplasm and they would give rise to dextral offspring. Now, in the hybrid $(s/+)$ half of the reduced eggs have a nucleus
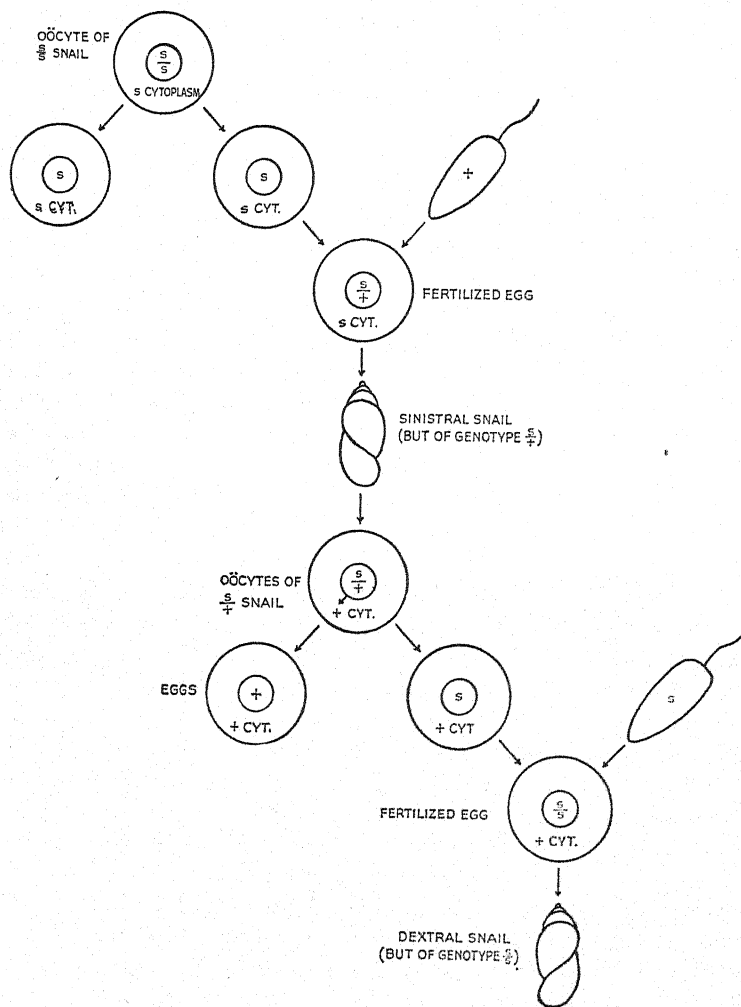
FIG. 146. The inheritance of coiling in snails.

of genotype s (Fig. 146) and if the s eggs were fertilized by s sperm, the resulting offspring (s/s) though pure for s would be dextral, because the egg cytoplasm was made dextral by the genotypic constitution of the hybrid mother ($+/s$).

Coiling in snails is often referred to as a case of "maternal inheritance," because the genotype of the mother determines the direction of coiling in the offspring. But the mother's genotype is determined by her father as well as by her mother, and so inheritance itself is really bi-parental, not exclusively maternal. It is therefore incorrect to refer to coiling as a case of maternal inheritance.

But the important point in the present connection is that the direction of coiling is not determined by hereditary bodies in the cytoplasm but by genes in the nucleus ($+$ and s). The only thing that is unusual about the case is that the genes exert their influence not on their own generation of developing larvae but on the next generation of larvae by their influence on the unfertilized egg from which the next generation develops. In other words, the effect of the genes is delayed.

The Inheritance of Plastids.—Many apparent cases of maternal inheritance are undoubtedly due to the delayed effects of genes, and such cases are not evidence for cytoplasmic inheritance. But in plants there is an important exception. The cytoplasm of plants contains minute bodies known as *plastids*. Certain of these produce chlorophyll, the green coloring substance in the leaves of the plant, and are known as chloroplasts. Plastids multiply by division in the manner of genes. Before a plant reproduces, a few plastids get into the eggs but none as a rule get into the pollen. Thus the offspring inherit their plastids solely from their mother.

Sometimes a seedling is white because its plastids lack chlorophyll. As a rule the white seedling is due to a recessive gene mutation which somehow prevents the chlorophyll from developing. The mutant gene is in a chromosome and is inherited like other genes; it might be transmitted to the offspring through either the pollen or the eggs. But sometimes the mutation is in the plastid itself, and in such cases it can be inherited only through the female line. An example of this sort is furnished by the four-o'clock, *Mirabilis jalapa*, studied by Correns. In four-o'clocks there is a race known as *albomaculata* in which the leaves are mottled with white and green patches. In the white patches all of the plastids are

without chlorophyll, but in the green patches at least some of the plastids are green, though not necessarily all. In fact, some green areas contain only green plastids; other green areas contain both green and white plastids. Between the boundaries of a uniform green and a white area, a cell often contains both kinds of plastids, and this suggests that in the developing plant the cells with both kinds of plastids give rise to the cells with only one kind. In fact, at cell division the plastids are distributed in a purely random manner, so that occasionally a cell gets only white or only green plastids. Such a cell might then give rise to a uniform patch by further cell division and growth. The size of a uniform area varies; it might include an entire branch. A seed from a uniform green branch gives rise to a seedling that is wholly green; and a seed from a white branch grows into a white seedling (which dies before maturity). In a mottled branch there are still large numbers of cells, including eggs, which contain both green and white plastids, and an egg cell with both kinds would upon being fertilized give rise to a mottled offspring. In any event, the source of the pollen would have no influence on the color of the offspring. Their color is determined entirely by the egg, and what the egg produces depends on the character of its plastids (whether green, white, or both). Since the plastids are in the cytoplasm, we are clearly dealing with a case of cytoplasmic inheritance. But apart from cases that involve plastids, we have no clear-cut evidence of cytoplasmic inheritance in either the plant or animal kingdom.

The Relation of Genes and Cytoplasm.—The fertilized egg contains cytoplasm in addition to genes, and it could not develop without both cytoplasm and genes. But the cytoplasm does not contain the units of Mendelian inheritance, and almost all of inheritance seems to be Mendelian. This would mean that chromosomes and genes are for the most part the physical basis of inheritance. They are the bodies that are transmitted from one generation to the next, and they determine development (in a given environment). They are often referred to as *determiners*.

Organizers, gradient-fields, and hormones are some of the important factors in development. But there is a time when none of these are as yet present in the early egg. Their appearance eventually is dependent upon the potentialities of the egg. In conclusion, then, the various possibilities of development reside within the egg itself and are dependent upon what is probably the most

highly organized material in the universe, the substance that constitutes the chromosomes and in particular the genes within them, the ultimate units of heredity.

SUMMARY

1. Genes control development; hence they cause the offspring to resemble their parents in a given environment.

2. All the cells of the developing embryo have the same kinds of genes. This is due to the fact that they all arise from the fertilized egg by mitosis.

3. Genes give a cell the capacity to develop in any one of a great many different directions. The particular direction in which a cell then develops is determined by the environment. The different parts of an egg or of an embryo differ in position, and their environments differ accordingly. Hence they develop in different directions. In other words, the environment causes *differentiation*. This account of differentiation is known as the theory of *epigenesis*.

4. In the frog's egg the first step in differentiation is the establishment of the long axis (from head to tail). This is due to the environment. For the frog's egg is undifferentiated at the very start of its existence (while it is still in the ovary). But one pole of the egg is nearer the arterial supply of the ovary, the opposite pole nearer the venous supply; and this difference in the environment of the two poles (or some such similar difference) causes the one to become the future head end and the other the future tail end.

5. Differentiation at first involves invisible chemical changes in the cytoplasm, referred to as *chemo-differentiation*.

6. The limb bud of a newt embryo can be removed and transplanted on the tail. If this is done at a certain stage (call it stage 1), the limb bud develops into part of the tail. If it is done somewhat later (stage 2), the limb bud develops into a limb (on the tail). Yet at stage 2 the limb bud cells are visibly undifferentiated and appear no different than at stage 1. But at stage 2 they are *chemically* differentiated, and this causes them to develop into a limb when transplanted on the tail.

7. In general, once a cell is chemically differentiated it tends to complete its differentiation and become visibly differentiated, even in some abnormal position.

8. Before chemo-differentiation, a cell can develop into any part of the embryo, if transplanted to the appropriate location. The embryo is said to be in the *plastic* stage of development.

9. Chemo-differentiation sets in gradually. The embryo becomes subdivided into smaller and smaller areas, each chemically differentiated

from the rest. When chemo-differentiation is complete, the embryo is said to be in the *mosaic* stage of development.

10. There is no sharp distinction between the plastic and the mosaic stage of development, since the first gradually changes into the second. The mosaic stage really begins with the first step in differentiation—the establishment of the long axis of the future embryo.

11. In some species the eggs are not chemo-differentiated to any great extent before fertilization; in others, they are. The first are said to be *indeterminate* eggs; the second, *determinate* or *mosaic* eggs.

12. If the first two cells of a developing sea urchin egg are separated both cells develop into a complete embryo; in the case of the snail's egg, they do not. The sea urchin egg is indeterminate, hence the first two cells are relatively undifferentiated and capable of developing into complete embryos. The snail's egg on the other hand is chemo-differentiated and therefore incapable of giving rise to two embryos.

13. In the early embryo some organs produce chemical substances which diffuse to neighboring cells and cause them to differentiate. Such organs are known as *organizers*, and their influence on neighboring organs is referred to as *induction*. The optic cup is an example of an organizer. It causes the cells in the ectoderm opposite it to become differentiated into a lens.

14. In the newt embryo the most important organizer is the *dorsal lip*. This folds in under the ectoderm and induces the formation of neural folds in the overlying ectoderm.

15. The dorsal lip is not evidence against the influence of genes on development, for genes give the ectoderm its capacity to produce neural folds, and the dorsal lip simply calls forth this capacity.

16. A *field* is a chemo-differentiated region of the embryo. Developmental activity is highest at some one point in a field and becomes gradually less with increasing distance from this point. In other words, there is a *gradient* of activity in every field.

17. Oxidations are one of the main evidences of developmental activity. There is a gradient of oxidation in every field, corresponding to the general gradient of developmental activity.

18. The long axis is the first field that is established in the development of the newt embryo. Oxidations are highest at the head end of the long axis and become gradually less toward the tail end.

19. In the mosaic stage of development each chemo-differentiated region is a sub-field.

20. A field and a gradient are inseparable and are jointly referred to as a *gradient-field*.

21. When an earthworm is cut into a number of pieces, each piece develops a new head at its fore end and a new tail at its hind end. This is due to the fact that the fore end of each piece is at a higher point than the hind end in the gradient of the long axis.

22. Twinning might be caused by anything which depresses activity at the head end of the axial gradient, thus causing two secondary high points to be established somewhat further back on each side of the midline.

23. Hormones have an important influence on development. Especially important in the vertebrates are the secretions of the thyroid, the pituitary and the gonads (ovaries and testes).

24. The thyroids produce thyroxin, a chemical substance which contains iodine and which increases the rate of chemical activities in the body, especially oxidations. Thyroxin causes metamorphosis of tadpoles. In man it causes oxidations to proceed at the rate necessary for normal development, both mental and physical. Cretinism is the result of thyroid deficiency.

25. The pituitary stimulates all the other glands that produce hormones. Thus, for example, the pituitary produces hormones that stimulate the growth and activity of the sex glands and that are necessary for normal sexual development. The pituitary also produces hormones that are necessary for growth in general. Underactivity of the pituitary might cause infantilism; overactivity, gigantism.

26. In the case of insects it has been demonstrated that hormones are under gene control. In the flour moth a mutation (red) has caused the loss of a hormone necessary for the development of the black pigment of the eye. This hormone is produced by the brain, the ovary, and the testis, and its development is dependent on the normal allele of red.

27. In Drosophila there are at least two hormones, *A* and *B*, necessary for the development of the normal red eye color. Normally, *A* is formed first and *B* cannot be formed unless *A* is present. A vermilion fly cannot form *A* and therefore ordinarily cannot form *B* either, but it can form *B* if *A* is supplied. A cinnabar fly cannot form *B* but can form *A*. Hence when a vermilion eye disk is transplanted into a cinnabar fly, it develops the normal red color because the cinnabar host supplies *A* and vermilion then makes *B*. But a cinnabar disk in a vermilion host remains cinnabar, because cinnabar cannot produce *B* and the host does not supply it (since it lacks both *A* and *B*).

28. The above experiments show that in Drosophila the normal allele of vermilion is necessary for the production of hormone *A*, since a vermilion fly cannot produce *A* but a normal fly can. Likewise, the normal allele of cinnabar is necessary for the production of hormone *B*. Thus we have evidence in Drosophila that hormones are under gene control.

29. Hormones are not evidence against the influence of genes on development, because hormones themselves are under gene control.

30. Genes often produce their effects by influencing the rate of development.

31. Crosses between species do not support the theory that the cytoplasm determines species differences and the chromosomes only varietal

differences, for when different species of echinoderms are crossed, the larva develops paternal as well as maternal traits, although it often develops the paternal traits somewhat later in development because it takes time for the paternal chromosomes to exert their influence on the cytoplasm.

32. In the fresh water snail (Limnaea), the gene for sinistral shell (s) and its normal allele ($+$) exert their effect, not on their own generation of developing larvae, but on the next generation by their influence on the cytoplasm of the unfertilized eggs from which the next generation develops. Thus, an s/s snail produces eggs with sinistral cytoplasm, and these develop into sinistral offspring even though they are fertilized by dextral sperm. But the offspring ($+/s$) produce eggs with dextral cytoplasm ($+$ being dominant) and therefore all *their* offspring are dextral. Thus the $+$ gene is not exerting its effect on the immediate offspring ($+/s$) but on the offspring of the next generation through its delayed effect on the cytoplasm of the eggs produced by the $+/s$ offspring.

33. When the effect of genes is delayed, the father does not have any immediate influence on the cytoplasm of the developing egg in crosses between species, and therefore it superficially appears as though the cytoplasm was determining development.

34. Plastids are bodies contained in the cytoplasm of plants, some of which produce chlorophyll (the green coloring substance in plants). Plastids multiply by division in the manner of genes, and they are usually transmitted to the offspring through the cytoplasm of the egg. They represent the only known case of cytoplasmic inheritance.

35. In a certain race of four-o'clocks (the *albomaculata* race) the leaves are mottled with green and white patches. A mottled plant develops from a fertilized egg which contains both green and white plastids. But in the course of cell division a cell sometimes receives only white plastids, and such cells give rise to the white areas. A cell sometimes receives only green plastids and gives rise to a pure green area. Most cells continue to have both kinds of plastids.

36. All well-established cases of inheritance are Mendelian, apart from the inheritance of plastids. It follows that the chromosomes are the physical basis of inheritance (with the exception noted). Hence the genes determine development. Organizers, gradient-fields, and hormones are factors in development, but they themselves are under gene control.

PROBLEMS

1. According to an older theory—the "preformation" theory—the adult was preformed in the unfertilized egg in miniature and contained all the organs of the adult, including an ovary with eggs. What would these eggs in turn contain, if the theory were true?

2. According to August Weismann, a nineteenth-century biologist, the chromosomes of the egg contained determiners for the nose, eyes, ears, and other organs of the body, and as cell division took place the determiners for each organ were sorted out. Tell how the chromosomes divide at cell division, and tell why this method of division would prevent the sorting out of determiners, postulated by Weismann. Tell also how we know that determiners are not sorted out in the first and second division of the sea urchin egg.

3. A pure sinistral snail (s/s) is crossed to a pure dextral ($+/+$). Give the appearance of the F_1 and the F_2 with the reason in each instance. Give the phenotypes of the F_3 if the F_2 are self-fertilized.

4. Hadorn fertilized the egg of one newt (*Triton palmatus*) with the sperm cell of another (*T. cristatus*) and then removed the "palmatus" nucleus from the egg before nuclear fusion. The egg thus treated does not develop to the adult stage, but it does develop to the stage when the skin cells begin to form, and these, if grafted upon the larva of another kind of newt, develop the coloration typical of *T. palmatus*, although they lack the palmatus nucleus. Tell how this result might be explained without the assumption of cytoplasmic determiners, bearing the snail case in mind.

5. If the cytoplasm contained hereditary units or "determiners" and if one egg were hybrid for two different kinds of cytoplasmic determiners, would such determiners segregate from each other at the reduction division in the way that alleles do? Tell why or why not.

6. What bodies in the cell segregate at the reduction division in the same manner as Mendel's units? What bodies therefore are probably the physical basis of Mendelian inheritance?

7. Numerous cases of Mendelian inheritance have been demonstrated in animals, but no clear-cut case of non-Mendelian inheritance has as yet been found. What kind of bodies would this fact indicate as being the physical basis of inheritance?

8. If all inheritance in animals is Mendelian, then upon what bodies, present in the fertilized egg of a newt, is the capacity to form a dorsal lip dependent?

9. Would you say that the discovery that the dorsal lip was an organizer in the newt embryo disproved that genes and chromosomes were the physical basis of inheritance? Tell why or why not.

10. Is the dorsal lip itself present in the fertilized egg of the newt? Would you say then that the dorsal lip is to be classified as germ plasm or as a trait?

11. Upon what two things is the development of any trait dependent? Would this be true of the dorsal lip in particular?

12. Conklin has found that the eggs of certain marine animals are differentiated into several zones, each containing a characteristic kind of

pigmented granule upon which the development of the muscles, noto-chord, and other organs are dependent, as shown by experiments in which the granules are shifted into abnormal locations (by centrifuging the eggs) and in which the organs in question then develop in correspondingly abnormal positions. Might the granules in question be the products of an early chemo-differentiation of the egg? If so, then what would have caused their chemo-differentiation? Would these granules, then, have been transmitted unchanged from one generation to the next like genes? Tell then briefly why the experiments in question are not necessarily evidence for cytoplasmic determiners (comparable to genes).

13. It is possible to separate the first eight cells (or blastomeres) of the developing sea urchin egg, and if this is carefully done, each blastomere will develop into a complete sea urchin. In the case of the newt or frog, the first two blastomeres can be separated and made to develop into two complete embryos but not the first four or later blastomeres. In still other cases (snails) the first two blastomeres if separated will develop only into half embryos, and then disintegrate. Tell how we might account for the differences in the "toti-potency" of the early blastomeres of these species, bearing in mind the fact that chemo-differentiation might start at different stages in development in different species.

20. *THE GENETIC BASIS OF EVOLUTION*

EVOLUTION has been defined in various
ways. In its most general sense it means simply a process of slow
and gradual change. A star gradually cools down and can be said
to undergo an evolutionary change. When we apply the term
evolution to living things we mean the gradual change of a species.
But a species does not have a continuous existence in the sense
that a star does. Instead it represents a succession of individuals
related as parents and offspring. When we speak of the evolution
of a species, we mean that the species becomes gradually changed
or modified in the process of descent. Briefly, then, we can define
evolution as *descent with modification.*

The Method of Evolution.—There can hardly be any reason-
able doubt but that species have had their origin through the
process of evolution. Fossils and stratified rocks give us almost
indisputable evidence to this effect. But just how did the process
of evolution come about? Did the horse, for example, become
speedier because its ancestors for many generations developed
speed through running? Or did it change to the swifter type through
the unfolding of some inner tendency, as when a child grows up
and becomes capable of walking and then running? These are
only two of several possibilities that have suggested themselves
to those who have thought of the problem. They are interesting as
speculations. But let us confine ourselves for the present to facts.

Evolution is tied up with reproduction, because evolution is
descent with modification, and descent involves reproduction. But
reproduction in turn is tied up with inheritance. Therefore, before
we can understand how evolution takes place, we must know
something about the process of reproduction and inheritance. We
must also know something about the process of variation, because
it is through variations that a species becomes modified in the

process of descent. Genetics concerns itself with the study of heredity and variation, and therefore it deals with the processes that are at the *basis* of evolution.

From a study of genetics we learn that some variations are inheritable, others are not. Only those that are inheritable can take part in evolution. Mutations are one kind of variation; they are inheritable. The differences that we see in the coat color of a litter of pups is another kind of variation; they too are inheritable. But they do not represent new mutations. They are due to the Mendelian recombination of old mutant genes for which the parents were hybrid. Still another kind of variation is represented by the differences in the size of the seeds gathered from a single bean plant (assuming that no mutation has occurred). These differences are due to the direct influence of nourishment and other environmental agents (not to a change in the germ plasm); they are not inherited.

It has been found that mutations occur in all forms of life which have been closely observed. They segregate from their normal alleles without contamination. Therefore, even though the mutants are at first isolated individuals and can reproduce only by breeding with normals, they do not revert to the normal by repeated contamination. This Mendel proved. Moreover, mutations which have arisen in separate individuals may be brought together by crossing and new races pure for the mutations may then come into existence through Mendelian recombination. But mutations are random in character, in the sense that most of them are changes for the worse, and only occasionally does one happen to be good. Therefore mutations in themselves could not cause evolution to proceed along *adaptive* lines. But in a state of nature bad mutations, such as idiocy, would be weeded out in competition with the good, and only the good would survive. This is *natural selection*. Basically evolution is not adaptive because it is dependent upon mutations. But it *becomes* adaptive through natural selection. In brief, the evidence from genetics indicates that adaptive evolution came about through mutations and natural selection. But other views are still held as to what the method is. In this connection it should be emphasized that evolution itself is a fact based directly on the fossil evidence. It remains a fact regardless of how it came about. Anti-evolutionists have contended that evolution is a disputed theory because biologists are not agreed as to its method.

This contention obviously represents a confusion of ideas. Evolution is one thing; its method quite another. We shall now consider theories other than the one above given as to the method of evolution.

Lamarckism.—According to a view sometimes held, a child can inherit the increased muscular strength of his parents if they acquired it through exercise; or their darker skin, if they acquired it through tanning by the sun; and so on. In other words, the view in question holds that *acquired traits* are inherited. If they really are, then evolution can take place through an inheritance of acquired traits. The theory that evolution came about in this manner is sometimes referred to as *Lamarckism*, having been named after Lamarck (1744–1829), a French naturalist. Lamarck deserves much credit for attempting to explain evolution at a time when the fact itself was not yet accepted by most biologists. But modern students of heredity do not believe in an inheritance of acquired traits because the evidence is opposed to it, as derived from two sources: (1) the study of mutation and (2) the pure line work.

The Evidence Against Lamarckism from the Study of Mutation.—Before the chromosome theory of heredity had been established, it was conceivable that acquired traits might be inherited, provided one postulated a theory suitable for the purpose at hand. Darwin, for example, assumed that all parts of the body sent particles (pangens) to the reproductive cells and that each part sent its own special kind of particles, as muscle particles, brain particles, and so on. The more a given part was developed, the greater was the number of particles supposedly sent from that part to the reproductive cells. In short, the reproductive cells were regarded as a sort of little house of representatives. According to this theory it is not difficult to see how acquired traits might be inherited.

But once it was discovered that inheritance took place through chromosomes, then it became evident that the inheritance of acquired traits was a virtual impossibility. For the chromosomes in a person's reproductive cells are not produced by the muscles, skin, and other parts of his body, but they are derived from the fertilized egg from which he develops. These in turn are derived from the previous generation through the reproductive cells of the person's parents, and they are transmitted to the next generation following him through his own reproductive cells. Moreover,

it was discovered that genes were the ultimate units of heredity, and that changes in type in a pure race took place largely through mutations in the genes.

It was therefore evident in the light of these newer discoveries that if an acquired trait were really to be inherited, a very peculiar series of processes would have to occur. The acquired trait would in the first place have to cause a mutation. As a rule, it would not do this. But it is conceivable that it might, in rare instances. For example, a tanned skin might conceivably produce some chemical substance that got to the reproductive cells and produced a mutation. So let us assume, for the sake of the discussion, that it did produce a mutation. When we say that tanned skin has produced a mutation we do not mean that it has produced a darker skin in the offspring but merely that it has produced a change in a *gene*.

But *now our difficulties begin*. For even if a tanned skin should cause a mutation—a changed gene—there is no known reason why the changed gene in turn should cause a darker skin to develop in the offspring; so far as we know, it would be just as likely to cause a taller size, or a smaller size, a stub nose, or red hair, or any other conceivable change. Mutations are *random* in nature. The effects which they produce bear no particular relationship to the agents which cause them. This becomes obvious when one remembers that X-rays are an efficient cause of mutations. Yet when X-rays cause a gene to mutate in a parent, the offspring that receive the mutation do not give out X-rays. The mutated gene produces some effect totally unlike X-rays. At present there is no known relationship between the cause of a mutation and the effect of the mutation on development. A given mutation would produce a definite effect, but what this would be we have no way of predicting. If the cause of a mutation were an acquired trait, the mutation almost certainly would *not* as a rule cause the development of a new trait that *imitated* the acquired trait. This would be just as improbable as that a broken pane of glass should upon falling reassemble and assume the shape of the object that struck it.

In summary, then, an acquired trait could hardly produce a mutation which would *imitate* the acquired trait in its effect on the next generation.

The Evidence Against Lamarckism from the Study of Pure Lines.—In beans all differences between the seeds of a given plant are solely *acquired traits*. This follows from the fact that

all of the seeds belong to the same pure line and all differences between them must therefore be due to the environment. But it will be recalled that if we selected the largest seed on a bean plant and grew it, it would not produce seeds of larger size on the average than any other seed on the plant. This therefore shows that acquired traits are not inherited.

It would not be so easy to prove the same thing in man, for in us size differences are not due entirely to the environment. They are due partly to hybridity on the part of our parents, just as the color differences between the children in a mulatto family are due partly to the hybridity of their parents. Hence, in order to show that acquired traits are not inherited, it is necessary first to rule out the confusing effects of hybridity, and this can be done by the use of pure lines.

Some earlier experiments, previous to the work on beans, indicated that acquired characters were not inherited. Weismann cut off the tails of rats for many generations, but found that rats continued to be born with tails. We might have known this, even without Weismann's experiment. If mutilations could be inherited, we should all be deformed. For everybody could find somewhere in his ancestry some person who had suffered a deforming injury (previous to reproduction), if he went back far enough in his family tree. Thus the entire race would be deformed if mutilations could be inherited. Circumcision has been practiced by the Jews and others for many generations, yet without inheritable effect. Neither have the deformed feet of Chinese women been inherited, nor any of the mutilations practiced by many savage races generation after generation. However, it might well be argued that the body has some protective mechanism against the inheritance of mutilations but that nevertheless acquired traits other than mutilations might be inherited. Weismann's experiment is therefore not as decisive as the work on beans in disproving the inheritance of acquired traits.

The Lack of Positive Evidence for Lamarckism from the Study of Evolution.—The experimental work to date, then, gives us no real evidence of an inheritance of acquired characters. Still it might be contended that our experiments are not carried on over a long enough period to give us a measurable effect; that in the course of evolution races do eventually get dark in the tropics, fishes do lose their eyes in dark caves, and so forth. But cases of

evolution do not really furnish evidence for the inheritance of acquired traits. For these cases can be explained on the basis of mutation and natural selection. We prefer so to explain them, because mutation is fact, not theory.

It would require much space to analyze in detail all the observations that have been advanced in support of Lamarckism. A case that has been singled out by exponents of the view is that of the wart hog of Africa which leans on its knees in eating and which is born with calluses on its knees exactly where they would be developed through use. The close correspondence in appearance between the trait as acquired through use and as inherited suggests that the one has been the cause of the other. But it should be borne in mind that the body has a tendency to develop calluses at the joints and this tendency can be exaggerated and brought into expression as the result either of use or mutation. Hence the correspondence of the trait as acquired by use and as produced by mutation. What in the first place caused the predisposition of certain areas on the knees to produce calluses and how these areas came to be in just the right places is another question. After all, the occurrence of calluses in just the right place is no more remarkable than many other adaptations, when one remembers that evolution has produced such complicated structures as the eye, the internal ear, the human brain—all consisting of almost innumerable parts in the most exact working relationship to each other. If mutation and natural selection can produce such complicated structures, then surely they can produce the relatively simple one that consists of a callus most efficiently distributed on the knees, or rather of areas of skin that have a predisposition to form such a callus and that can be stimulated into actual callus formation either by pressure or mutation.

It is true that the facts just reviewed and some others of the same kind superficially suggest an inheritance of acquired traits. That is why the belief is so commonly held. But it is not supported by a critical analysis of the facts nor by our present-day knowledge of heredity.

The Theory of Orthogenesis.—We come now to a theory known as *orthogenesis*, according to which evolution tends to take place in certain directions (usually adaptive), determined exclusively by tendencies within the organism. For example, the theory claims that the ancestors of the horse evolved longer legs because

of some inner tendency on the part of their germ plasm to produce longer legs. It claims that mutations or their equivalent did not take place in a random manner and in all directions at each stage in evolution but in only one or a few directions, as determined by the inner tendency. Superficially, the theory seems to be supported by the fossil evidence. For a series of fossils such as the horse series is apt to give the impression that evolution takes place through orthogenesis, since the series seems to proceed directly to an end. In fact, those who believe in the theory claim that the fossil evidence supports it.

Fossils are very valuable in giving us the general course of evolution, but they can give us very little evidence of the precise *method* by which evolution came about. Take the case of the horse series. This shows beyond any reasonable doubt that the present-day horse arose from a simple ancestor through a series of intermediate steps. Nevertheless, each member of the series is separated from the next by a long period compared with the lifetime of a horse. Just what happened in each generation of horses, the fossils do not tell. Many mutations, of all sorts, might have taken place between any stage and the next one shown by the fossils. But only those that led to increased swiftness would have continued to live and multiply. The rest would never have become sufficiently abundant or distinct to be evident in the fossil remains. Thus the evolution of the horse might have been due to mutation and natural selection. In general, any series of fossils that is adaptive could be accounted for in the same way.

But palaeontology supplies us with cases of evolution which at first sight seem to support orthogenesis. In illustration we might take the titanotheres, an extinct group of mammals somewhat resembling a rhinoceros. There were four main lines of titanotheres. Each line not only evolved great bulk but it also evolved a curious V-shaped horn at the end of the nose. The four lines can all be traced back to a comparatively small ancestor which lacked the horn. It therefore appears that the hornless ancestor had a tendency to develop horns, and that each of the four lines developed horns because of this tendency. However, the horn was already present in the original ancestor in the form of a small nodule, and any mutation which increased the size of the animal might at the same time have increased the size of the nodule. In the horned animals in general, it is in fact found that horn size is

correlated with body size. Moreover, when growth takes place, horn size increases relatively faster than body size in most horned animals, so that if the body doubled its size, the horns would much more than double their size. A difference in the growth rate of two parts of the body is sometimes referred to as *heterogonic* growth. Hence any mutations which caused increased size of the body would cause a relatively greater increase in the size of the horns. In the later and larger titanotheres, the horns are of relatively great size, due undoubtedly to heterogonic growth.

In the evolution of snails it is found that the shell becomes more and more tightly coiled, though beyond a certain point the coiling is of no apparent advantage and might seem to be due to a tendency toward coiling present in the original snail stock. We do not definitely know the explanation of this case. Perhaps it is a case of orthogenesis. But perhaps we are again dealing with a case of heterogonic growth. The shell coils because the outer side grows at a faster rate than the inner side, and any mutation which caused a faster growth of the shell, itself of advantage, might incidentally cause increased tightness of the coils by causing a relatively greater growth rate of the outer side of the shell as compared to the inner side.

Then again, in the evolution of the saber-tooth tigers the two upper canines became so long as seemingly to obstruct the mouth opening. According to orthogenesis the early ancestors of the saber-tooth tiger had a tendency to produce long canines, and this tendency expressed itself despite its harmful consequences to the species. But careful anatomical studies have shown that the saber-tooth tiger could open its jaws very widely, in a way that would have enabled it to use its canines for stabbing thick-skinned prey. The canines would therefore have been very formidable weapons of attack and would have been of use to the saber-tooth tiger. Hence their evolution might be explained by natural selection. What it was that caused the saber-tooth tigers to become extinct is something we are not sure of. Perhaps it was the dying out of their thick-skinned prey in the region of the world that they inhabited.

The theory of orthogenesis totally fails to explain why evolution *did* proceed along adaptive lines. The mere assertion that adaptation comes about through an inner tendency is about as much of an explanation as that a locomotive engine moves because of

some inner tendency. Moreover, when we study *existing* forms of life, we see no conclusive evidence in favor of orthogenesis. Mutations take place in *all* directions, not just in one direction.

Summary of Theories of the Method of Evolution.—In summary, three theories have been considered regarding the method of evolution: (1) the theory that adaptive evolution comes about through mutations and natural selection, (2) the theory that it comes about through an inheritance of acquired traits (Lamarckism), (3) the theory of orthogenesis. The evidence from genetics favors the first of these theories. For mutations have been observed to take place naturally, in a large number of species, and they have also been artificially produced. The evidence indicates that evolution is dependent on mutations and is therefore basically a random process, but that it becomes exclusively or almost exclusively adaptive in the long run because the non-adaptive mutations die out; that is, because of natural selection.

The Spread of Genes Independently of Natural Selection.—In man blue eye color has no particular advantage over brown; yet it has become fairly common. How does it happen that mutants of no particular survival value often spread and get established?

A gene might become relatively abundant purely through accidental spread. This could happen especially in small isolated populations. Take an extreme case where a population consists at the start of just two individuals, one a pure normal $(+/+)$ and the other hybrid for the mutant gene a $(a/+)$. The offspring would on the average be $1 +/+ : 1 a/+$, but as a matter of chance both might be $a/+$. In this case the mutant gene would have increased from 25 per cent of the total (when the population was $+/+$ and $a/+$) to 50 per cent of the total (when the population became $2 a/+$). Moreover the hybrids $(a/+)$ would on the average throw offspring in the ratio of $1 +/+ : 2 a/+ : 1 a/a$, but if they threw just two offspring then obviously this ratio could not be realized. If one of the two offspring happened to be $+/+$ and the other a/a, or if both happened to be hybrids $(a/+)$, then 50 per cent of the genes would still be $+$ and 50 per cent a. But more often the two offspring would belong to other possible combinations of the three Mendelian classes (such as both a/a or one a/a and the other $a/+$), and in this way the mutant gene a might become more abundant (though the normal gene might instead). The genes in a population so small would in fact tend to consist

eventually either of + or of *a*, and it would be a 50–50 chance which of these two genes it would be, if the population were originally *a*/+, and if the mutant gene were neither bad nor good.

As a rule a natural population would not be as small as the one here selected as an illustration, but the same general outcome would apply to a larger population provided it were not too large. The population might now get very much larger for some reason or other and the gene which had spread throughout its members when it was small would then become correspondingly more abundant. Natural selection was not involved in the spread of the gene in question, for by natural selection we mean the survival of the fittest, and the gene in question did not survive because it was fittest but because of accident. The accidental spreading of a gene is sometimes referred to as *drift*.

A gene might become more abundant through the process of mutation itself. Suppose, for example, that gene + mutated to *a* at certain rate. Then if *a* did not change back to + and if it were not eliminated by natural selection, the whole of + would eventually change over to *a*, although this might take a long time. But suppose *a* did change back to + (by reverse mutation) and that we began with nothing but +'s. Then at first the *a*'s would accumulate in the population and this would continue for a while. But in the meantime some of the *a*'s would be lost by reverse mutation, and the number lost would continue to increase as the number of *a*'s themselves increased (since the greater the number of *a*'s the greater would be the number of reverse mutations). Eventually a point would be reached where as many *a*'s were lost by reverse mutation as were gained by forward mutation from +. At this point the two genes + and *a* are said to be in a state of *equilibrium*. In other words, neither is now gaining or losing in numbers; or rather each is gaining as much as it is losing per unit time.

What will be the relative amounts of + and *a* when they are in a state of *equilibrium*? This will depend on the relative rates of mutation and reverse mutation. If the reverse mutation (*a* to +) takes place, let us say, just half as frequently per unit time as the mutation from + to *a*, then obviously it will take twice as many *a*'s to form a given number of +'s per unit time, as it will take the +'s to form the same number of *a*'s. In other words, when the ratio of the two genes is 1 + : 2 *a*, then the population will be

in a state of equilibrium with respect to the two genes in question. This state would have been arrived at without natural selection, since it is being assumed that the mutant gene *a* has no particular survival value. We can speak of the continued production of a gene by mutation as *mutation pressure*.

In summary, then, genes having no particular survival value might get established in a population through (1) accidental spread (drift) and (2) mutation pressure. In this way the population might become diversified. The mutant genes themselves would have no particular relation to one another in the sense that they all modified an organ in some particular direction. In evolution, however, mutations are usually piled up in some particular direction, and the direction itself is *adaptive*, as when a series of mutations leads to increased speed. Now, long-continued evolution along adaptive lines could come about only through natural selection.

But the process of natural selection could be greatly assisted by the two methods of genic increase mentioned above. A mutant gene might by itself have just slight survival value and it would be very difficult for such a gene to spread through natural selection, especially at the start. For instance, in the tropics a mutation that made for a slightly darker skin color would have a slight advantage over the lighter type, but it is not likely that this slight advantage would immediately result in its possessor having more offspring than lighter skin-colored people have. If, however, the gene first spread through a small community by accident, then it might get established and become fairly abundant. At this point natural selection might step in and become a potent factor in the further spread of the gene, for now the darker skinned type would *on the average* have an advantage over the lighter and might tend to displace the lighter because of this average advantage, especially in conjunction with other slight advantages which might also at first have spread without the help of natural selection. Sexual reproduction and Mendelian recombination would bring together such slight advantages.

It is not to be thought that natural selection would mean that all the descendants of the lighter type would be weeded out and only those of the darker type survive. The lighter type would also have produced its quota of good mutations, and these might have spread at first without the aid of natural selection. But

once the mutants had become fairly abundant, sexual reproduction would be effective in interweaving the two germ plasms and bringing into existence favorable combinations of genes on a relatively large scale. These *combinations* would be definitely favored by natural selection; that is, they would tend in the long run to spread and displace the less favorable combinations. Neither the dark nor the light types would have been exclusively the ancestors of the population as it now exists. Both types would have contributed their quota of good genes to the evolution of the race.

Geographical Isolation.—Evolution has been compared to the branching of a tree. It results in diversification—the end twigs of the tree. The process of diversification as a rule results from adaptation to diverse environments and comes about through natural selection. But often a species is diversified in non-adaptive directions. This is especially true of species which live in small geographically isolated groups, as on different islands. For example, in the Galapagos Islands (off South America) each island is inhabited by a different variety of tortoise or by a different variety of mocking bird. The environment is not very different from one island to another, and it is therefore highly improbable that each variety of tortoise or mocking bird is especially adapted to its own island. Then just why did they become different? For one thing, geographical isolation breaks up a species into smaller groups and so it assists in the spreading of mutant genes by accident. Moreover, the same mutant genes would not necessarily arise and spread in any two groups. Thus each group would become different from every other one—the species would become diversified. Isolation would then maintain diversification by preventing one group from crossing with another. Geographical isolation might also lead to diversity by reducing competition and allowing inferior types to survive. This is well seen in Australia where the lower types of mammals— the marsupials—survived because they were protected from competition with the higher mammals by Australia's isolation.

Ecological Isolation.—Geographical barriers are not the only means by which animals or plants might be isolated from one another. One race might be able to tolerate a higher temperature than another, and so the two might occupy different localities. They might also be separated by differences in the moisture they can tolerate, by differences in the kind of food they eat, and by other differences of an ecological nature.

Interspecific Sterility.—If different species of animals and plants had been able freely to interbreed and produce fertile offspring in the past, no two species would today be distinct from each other, but instead all would form one conglomerate group in which any two extremes would be connected by intermediates, as happens for example when the Negro and white races interbreed. It therefore follows that before the members of a species can become differentiated from one another and form new species, they must somehow be prevented from interbreeding. This has often come about through geographical or ecological isolation. But when two closely related species occupy the same geographical region, they might be prevented from mixing either because they do not cross with one another, or if they do cross they produce sterile offspring as when the horse and donkey produce a mule. The production of sterile offspring by parents belonging to different species is known as *interspecific sterility*.

What causes interspecific sterility? It will be recalled that the radish and the cabbage can be crossed but that the chromosomes are so dissimilar that they do not pair in the diploid hybrid. Therefore the hybrid cannot produce normal gametes and is sterile. Chromosome dissimilarity, then, is one possible cause of hybrid sterility. But sometimes the chromosomes of two species are fairly similar and yet the hybrid is sterile. In this case the sterility is due most likely to mutant genes that make for sterility. But it would be rather difficult for a single mutation to cause hybrid sterility. For assume first that the mutation is dominant (A). Then the hybrid formed by crossing the mutant type (A/A) by the recessive parent type ($+/+$) would be $+/A$, and phenotypically this would be no different from the mutant (A/A), since A is dominant. Hence if the mutant gene A makes the hybrid sterile ($+/A$), it will also make the mutant itself (A/A) sterile, and thus incapable of reproducing its own kind. If on the other hand the mutant gene is a recessive (a), then the hybrid ($a/+$) will be no different phenotypically from the normal parent (the non-mutant, $+/+$), and therefore the hybrid would not be sterile.

But suppose that a recessive mutation (a) should occur first, and next a dominant mutation (B), so that the composition of the homozygous double mutant was $\dfrac{a}{a}\dfrac{B}{B}$ and of the pure normal $\dfrac{+}{+}\dfrac{+}{+}$. Suppose further that a made it possible for the species to

have B without being sterile (that is, the combination aB is not sterile), but $+$ and B in combination $\left(\dfrac{+\ B}{+\ B}\right)$ did produce sterility. Then the double mutant $\dfrac{a}{a}\dfrac{B}{B}$ would be fertile; so would the normal $\dfrac{+\ +}{+\ +}$. But the cross of the two $\left(\dfrac{a}{a}\dfrac{B}{B} \times \dfrac{+\ +}{+\ +}\right)$ would produce a hybrid of genotype $\dfrac{a}{+}\dfrac{B}{+}$, and since this contains both $+$ (the normal allele of a) and B, it would be sterile. We can refer to the genes $+$ and B as *complementary genes* for sterility. Interspecific sterility then might be due to complementary genes.

A case of hybrid sterility due to complementary genes has been found in *Drosophila pseudo-obscura* by Dobzhansky. In this species there are two races, named A and B, which do not differ much in appearance. When the two races are crossed the male offspring are sterile. But the female offspring are not, and they can be crossed to either parent race. They then produce some fertile male offspring, but the ratio in which they produce them indicates the F_1 female parent was hybrid for at least three pairs of genes and only those offspring which receive all three genes of race A or all three of race B are fertile. This means that an off-spring is sterile if it has any one of the genes of race A together with any one of race B. In other words, any two such genes in combination produce sterility. This makes them complementary in the production of sterility.

When hybrid sterility is due to complementary genes, it might express itself in the failure of the reproductive organs to develop. But sometimes the gametes are not properly formed, though the reproductive organs themselves are otherwise normal. In such cases the chromosomes might not pair at the reduction division because the complementary genes somehow prevent them from pairing.

But in the case of the radish-cabbage hybrid the chromosomes failed to pair because of chromosome dissimilarity. How do we know that their failure to pair was not due to complementary genes? It will be recalled that the radish-cabbage hybrid some-times produces an allotetraploid (containing two sets of radish chromosomes and two sets of cabbage chromosomes), and that chromosome pairing takes place in the normal manner in the

allotetraploid. We therefore know that complementary genes are not preventing the pairing of the chromosomes, and that their failure to pair in the ordinary diploid hybrid must be due to chromosome dissimilarity. It is stated that the sterility of the mule is due to the unlikeness of the horse and donkey chromosomes which enter into the formation of the mule, but it is not at present possible to get an allotetraploid mule and prove this. It is probable that most interspecific sterility, especially between species which are to outward appearance very much alike, is due to the action of complementary genes, not to chromosome dissimilarity. For it would take a good many mutations to make the chromosomes sufficiently dissimilar to prevent synapsis.

Once two closely related races had become sterile with one another, by whatever method, then they would tend to evolve rather rapidly in different directions, even though they occupied the same geographical area. For now the two races would tend to accumulate their own mutations because of their genetic isolation, and so they would eventually become very different in outward appearance. Thus interspecific sterility would be a very important factor in the differentiation of species.

As a rule the members of a given species are fertile when bred with one another but sterile when bred to some other species. This rule does not always hold, but the exceptions are relatively few. Thus sterility serves as the most practical means of defining the limits of a species. From what has been said above, it is obvious why this should be so, for species have often become different from one another because of the very fact that they are sterile with one another.

It would, however, be difficult for a mutant race to spread if it were sterile with the parent race, because at the start of its existence it would not be abundant and it would often breed with the parent race. The resulting sterility would prevent it from increasing in numbers. But the mutant race might at first be isolated from the parent race in one way or another. It could then breed with itself and become established.

Chromosomal Rearrangements in Evolution.—It will be recalled that Datura is differentiated into a number of geographical races known as prime types each having chromosomes with a different segmental arrangement as a result of translocations. Then again in *Drosophila pseudo-obscura* races occupying different

regions of California and Mexico differ in the segmental arrangement of their chromosomes and most of these races are derivable from a normal or a standard race by inversions.

It is evident from these and other cases that chromosomal rearrangements have occurred in the course of evolution. But they probably have not been as important as point mutations for evolution. Adaptations can arise only through mutations which produce changes for the better, but mere rearrangements often have no effect on development, either for the good or bad. This applies especially to translocations and inversions. However, there is occasionally a "position" effect.

Changes in Chromosome Number in Evolution.—Species might become differentiated through changes in chromosome number. In the plant kingdom tetraploid races have arisen through the doubling of the chromosome number. Higher polyploids have arisen by further doubling.

Increase in chromosome number probably never comes about by mere breakage of chromosomes into two or more segments. For when a chromosome breaks into two, one of the fragments is left without a spindle fiber attachment and this fragment would get lost in the process of division. Then, again, chromosomes with broken ends cannot survive. For both these reasons the chromosome number could not be increased by simple breakage. Neither could it be decreased, say, from eight to four, by the end-to-end union of entire chromosomes in two's. For only broken ends of chromosome segments can unite, not intact ends. Then, too, the union of two entire chromosomes would result in a chromosome with two spindle fiber attachments, and such a chromosome would get lost in the process of cell division, because at metaphase it would often be drawn to opposite poles of the dividing cell and so get stuck in the middle.

The chromosome number might, however, be decreased. Thus in Fig. 147, upper part, we begin with two chromosomes each having a sub-terminal spindle fiber attachment (that is, an attachment near but not at the very end of the chromosome). The region of each chromosome immediately to either side of the spindle fiber attachment is often inert, and this is indicated by lighter shading in the figure. One chromosome is arbitrarily divided into segments *A* and *B*, the other into *C* and *D* (in Fig. 147, upper part). Suppose now that each chromosome should become broken

into its segments, and suppose further that segments *A* and *D* should become attached, and segments *C* and *B*. Then two new chromosomes would be formed; namely, *AD* and *CB*. An offspring which received *AD* would survive, even though it did not receive *CB;* first, because *AD* contains all the active chromatin and second, because *AD* has one and only one spindle fiber attachment. In *effect*, then, the two chromosomes have become united end on,
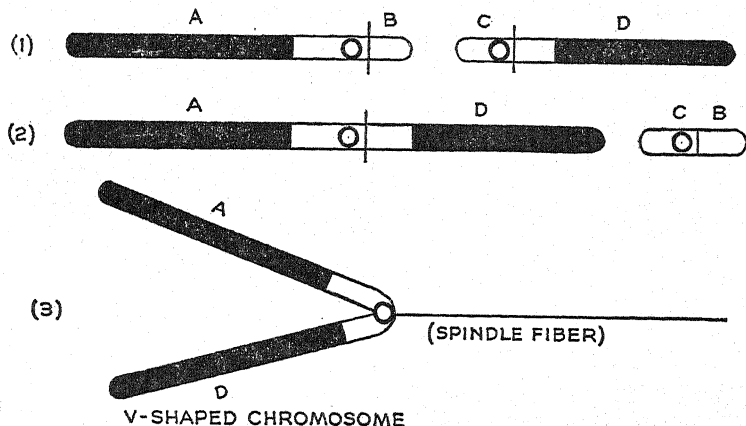
Fig. 147. A decrease in chromosome number through translocation.

and the chromosome number has been decreased, through a translocation. The spindle fiber attachment now is median, and this results in a V-shaped chromosome (Fig. 147 bottom).

Drosophila melanogaster contains a pair of rod-shaped chromosomes (the *X* chromosomes, in the female), two V-shaped pairs, and a dot-shaped pair (Fig. 148, left, which shows just one member of each pair). Originally the two arms of each V-shaped chromosome were probably separate chromosomes and were rod-shaped, as seen in *D. virilis* and in several other species of Drosophila (Fig. 148, middle). We can, for the sake of brevity, use the term "arms" in referring to the rod-shaped chromosomes that correspond to the arms of the V-shaped chromosomes in *D. melanogaster*. In brief then, the original condition is supposedly a pair of rod-shaped *X*'s, four separate pairs of arms, and a small fourth pair, making six pairs in all; or, if we refer to the haploid number, there are originally six chromosomes, consisting of an *X*, four separate arms, and a small fourth chromosome.

In some species of Drosophila just two of the arms are united, resulting in one V-shaped pair. In others the two remaining arms also are united, giving two V-shaped pairs, as seen in *D. melanogaster*. Sometimes one of the arms is united with the X (by translocation), giving a V-shaped X, but leaving one less autosomal arm (Fig. 148, right). Again, one of the arms might be united with the short fourth chromosome, apparently obliterating the fourth chromosome. And so on.

Chromosomes always arise from pre-existing chromosomes; they never arise anew from non-chromosomal material. But we know

MELANOGASTER   VIRILIS   PSEUDO-OBSCURA
SIMULANS   SUBOBSCURA   MIRANDA
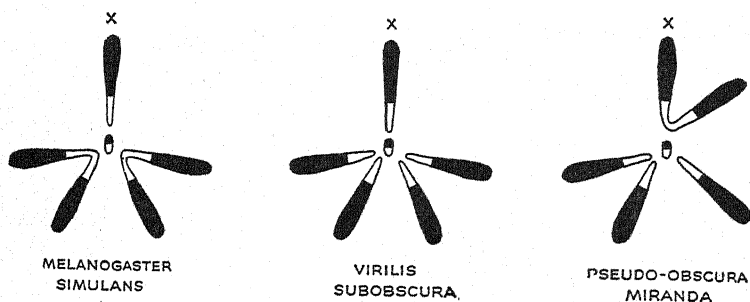
FIG. 148. Chromosome numbers in various species of Drosophila.

that in the course of evolution new chromosomes must have come into existence, for species differ in the number and kind of chromosomes they contain. How then do new chromosomes come into existence? This we have already indicated. Chromosome number might be increased through duplication, as in polyploidy. The chromosomes might then become differentiated through inversions, translocations, and most important through point mutations, for these would be the biggest factor in species differentiation and in the evolution of adaptations.

The Gene as the Basis of Life.—How did genes come into existence in the first place? To answer this question we should have to know how life itself originated. The big gap between the living and lifeless worlds is partly bridged by viruses. Recently Stanley has been able to isolate the virus which causes the plant disease known as tobacco mosaic. This Stanley has done by mashing up the diseased tobacco leaves and separating the virus from a solution of mashed-up leaves by means of certain salts. The virus is precipitated out of the solution as a crystalline protein and is

known as *Stanley's protein*. Is this protein to be considered living or lifeless? It does not possess the complicated cellular organization that ordinarily goes with living substance. To all appearances it is like any other protein, and proteins in themselves are not as a rule alive. Yet Stanley's protein has a remarkable property in common with all other living substance. It has the power of *growth*. Inject the smallest conceivable amount of Stanley's protein into the leaf of a healthy tobacco plant, and in a short time the entire plant is infected and killed. The virus has grown and spread over the entire plant. It is alive, yet about the only noticeable property it has that entitles it to be considered alive is its power of growth. But that is enough. For *life probably originated about the power of growth*. Other properties of living substance were later developments in evolution,—such properties as movement, the conduction of impulses, and consciousness. Reproduction and heredity depend on growth. Reproduction in its simplest form is merely growth followed by division—two are made out of one. Heredity is the transmission of the detached products of growth from parent to offspring. That makes the offspring like the parents, in a given environment.

The part of the cell characterized by the power of growth and reproduction is the gene. If life originated about the power of growth, then it originated about the gene, and the gene is essentially the physical basis of life. If we knew how the gene arose we probably should know how life itself arose from the inorganic.

Perhaps viruses consist of naked genes. It is conceivable that at one time living matter consisted merely of naked genes. Next cells would have been formed, at first very simple like those of the bacteria without nucleus and chromosomes but with cytoplasm in addition to genes. Chromosomes would then have been formed from the genes. Thus typical cells would have come into existence. The further course of evolution would then have been marked by increased complexity of organization with the cell as the unit of structure and function.

SUMMARY

1. Evolution might be defined as *descent with modification*.

2. The stratified rocks with their contained fossils are strong evidence for evolution. But the stratified rocks do not tell us *how* evolution came about.

3. The evidence from genetics indicates that all heritable variations eventually come about through mutation. This evidence therefore indicates that mutations are the elementary steps in evolution.

4. Evolution usually takes place along adaptive lines. But most mutations are bad. Hence adaptive evolution can come about only through the survival of the good mutations. The weeding out of bad mutations and the survival of the good is *natural selection*. The study of genetics therefore indicates that adaptive evolution came about through the natural selection of mutations.

5. According to an older theory, a child can inherit the increased muscular strength acquired by his parents through exercise; or the darker skin acquired by his parents as a result of tanning; or any other acquired trait.

6. In general, an acquired trait is a modification due to use or disuse or to the direct action of the environment (without the intervention of a mutation).

7. Lamarckism is the theory that evolution came about through the inheritance of acquired traits.

8. The evidence from genetics indicates that acquired traits are not inherited. This evidence is derived from (1) the study of mutations and (2) the pure line work as described in paragraphs 9 and 10 below.

9. It is conceivable that a tanned skin, for example, might produce some chemical substance which might reach the reproductive organs and cause a mutation, but it is very unlikely that the mutation in turn would cause the offspring to develop a darker skin. Much more likely it would have some totally different effect. Hence mutations cannot *reproduce* acquired traits.

10. All the beans on a given bean plant belong to the same pure line. Hence all differences between them are acquired traits. But one of the heavier beans on a bean plant does not produce offspring beans that are heavier on the average than the average for its line. Hence acquired traits are not inherited.

11. Orthogenesis is the theory that evolution takes place in certain directions which are determined by tendencies within the organism. Fossils have been interpreted as evidence for the theory.

12. A series of fossils, such as the horse series, seems to proceed directly along one line, but this is probably due to the elimination of all mutations except those in the main line, the other mutations having taken place in all directions at each stage in evolution. Hence an ordinary series of fossils is not evidence for orthogenesis.

13. The three theories of the method of evolution are: (1) the theory of natural selection, (2) Lamarckism, (3) orthogenesis. The evidence from genetics supports the theory of natural selection.

14. In a small isolated population a gene might spread accidentally. This is known as *drift*.

15. If a gene was repeatedly produced by mutation, it would tend to become abundant. The continued production of a gene by mutation is known as *mutation pressure*.

16. Mutations which by themselves have just a slight advantage might become somewhat abundant through drift and mutation pressure. They might then be combined by crossing and Mendelian recombination. In combination they might have a decided advantage, and they might then spread by natural selection.

17. Geographical isolation causes a species to become diversified (1) by assisting in the accidental spread of mutant genes and (2) by reducing competition and allowing the inferior types to survive. Ecological isolation has a similar effect.

18. Interspecific sterility is the production of sterile offspring by parents belonging to different species, as the horse and the donkey. Chromosome dissimilarity, causing failure of pairing at the reduction division, is one possible cause of interspecific sterility. Complementary genes for sterility are another possible cause.

19. Interspecific sterility is one means by which species are isolated from one another. Isolation tends to make species evolve in separate directions.

20. A species is sometimes differentiated into geographical races which differ in the segmental arrangement of their chromosomes. But segmental rearrangements as a rule produce no visible effects, and therefore they are not as important as point mutations in evolution.

21. Chromosomes might be increased in number through polyploidy and they might then become differentiated through gene mutations and chromosomal rearrangements.

22. In the genus Drosophila the chromosome number has been decreased by translocations which have resulted in chromosome union.

23. The gene is the basis of life, because it has the capacity of growth and of reproducing itself in its changed form when it mutates. It was this capacity that led to the evolution of life.

PROBLEMS

1. Darwin argued that a new mutant would have bred with the normals and the mutant type would therefore gradually return to normal because of contamination. He therefore believed that evolution came about through small variations that were constantly taking place in any species, rather than through mutations. Tell why Darwin would not have been faced with this difficulty in connection with mutations, if he had known of Mendel's experiments. Tell also how his ideas in connection with variations would have been modified if he had lived in Johannsen's day.

2. Tell whether Darwin was right or wrong, in the light of present-day genetics, in stating (1) that variations were "fortuitous" (mostly bad, but occasionally good), (2) that the bad variations were weeded out in competition with the good in a state of nature, (3) that slight or "fluctuating" variations could, in all cases, be accumulated by selection and lead to evolution. Tell what evidence we now have bearing on (1) and (3). Would you say that (2) required experimental evidence before it could be accepted, or, that it was a conclusion based on logic?

3. Tell why beans are better material for testing the inheritance of acquired traits than corn.

4. Given a population initially of composition $1 A/A : 3 a/A$ (that is, a population containing three times as many hybrids as pure A's). Give the relative proportions of *gametes* of classes A and a in the population as a *whole*. Tell in what ratio the offspring would tend to be produced, if fertilizations were random. (*Hint:* Bear in mind the manner in which you make the combinations between the gametes when the parents are a/A, as when the F_1 in a simple Mendelian experiment are bred together, and in the present case make the combinations in a similar manner but make them between gametes in the present gametic ratio.)

5. In the above example would the population produce its gametes in the ratio you give if alleles did not segregate? What then ultimately causes the offspring to be produced in the ratio you give?

6. Tell why a recessive mutation would not necessarily be kept from expressing itself in a freely interbreeding population, even if it were outnumbered by the normals.

7. By "genetic equilibrium" we mean the ratio which Mendelian classes tend to come to in a freely interbreeding population and in the absence of selection. Given a population initially of composition $4 A/A : 7 a/A : 1 a/a$. Derive the composition of the population after genetic equilibrium has been attained, assuming there are no new mutations. (*Hint:* First get the gametic ratio in the population as a whole, and then make the combinations between the gametes, as in problem 4 above.)

8. Assume that gene A mutates occasionally to a ("forward" mutation) and that a occasionally mutates back to A ("reverse" mutation). Assume further that the rate of reverse mutations is three times that of forward mutations. Tell what the relative numbers of A and a would be when neither is gaining nor losing in numbers.

9. Tell why the evolution of life would have been impossible in the absence of death.

10. In your opinion, which of the possible applications of genetics might at some future time prove of the greatest value to man?

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